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LABORATORY SKILLS  
FOR  
PLANT OPERATORS

VOLUME 1  
PRECOURSE STUDY MATERIAL



Ontario

Environment  
Environnement



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LABORATORY SKILLS FOR  
PLANT OPERATORS

VOL. I

Training and Certification Section  
Personnel Services Branch  
Ministry of the Environment  
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## LABORATORY SKILLS FOR PLANT OPERATORS

### INTRODUCTION

The Laboratory Skills for Plant Operators workshop based on this manual covers five days at the Ministry of the Environment training facilities. The workshop consists of lecture-discussions and hands-on training in treatment plant-oriented laboratory skills.

The principle objective of the workshop is to provide plant staff with the skills required to carry out inplant testing and analysis.

The curriculum covers techniques, procedures and tests which provide information for day-to-day process control. A successful candidate will be capable of performing the laboratory tests in either a water or wastewater treatment environment, evaluating data and advising on actions required for process control.

Volume I of this manual contains pre-study material on basic chemical and lab technique principles. volume II contains detail on specific lab procedures.



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SUBJECT:

TOPIC: 1

BASIC CHEMICAL PRINCIPLES

**OBJECTIVES:**

The Student will be able to:

1. Select the correct definition from a given list for each of the following items:
  - a. electron
  - b. proton
  - c. neutron
  - d. atom
  - e. element
  - f. compound
  - g. valence
2. Select the examples from a given list to indicate the following terms:
  - a. ion    i)        anion  
          ii)       cation
  - b. radical
  - c. organic compound
  - d. inorganic compound
3. Calculate molecular weight for specified compounds given a list of atomic weights.
4. Select from a given list, the correct name for a given simple compound.



LABORATORY SKILLS  
BASIC CHEMICAL PRINCIPLES

CHEMISTRY

Chemistry might be defined as the science that deals with the composition, properties and changes undergone by matter under certain influences.

MATTER

Matter is defined as anything that occupies space and has mass.

The page these words are written on is a form of matter, as is the ink which forms the letters on the page.

Some forms of matter consist of a single kind of matter called a pure substance.

ATOMS

Pure substances are made up of one or more "atoms". An atom might be defined as the smallest particle we could separate which would still exhibit the characteristic properties of that pure substance.

Pure substances can be either "elements" or "compounds". Elements are Pure Substances which contain only one kind of atom, whereas compounds are pure substances which contain more than one kind of atom.

Examples of common elements are gold (Au), chlorine (Cl), sulphur (S), oxygen (O) and hydrogen (H).

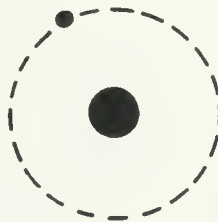
Examples of common compounds are sodium chloride (NaCl), water (H<sub>2</sub>O), hydrogen sulphide (H<sub>2</sub>S) and calcium hydroxide (CaOH).

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IIIA IVA VA VIA VIIA GASES

1-2

## ELEMENTS

We have established that matter is composed of one or more elements, but what is an element? An element is a pure substance which may be a solid, a liquid, or a gas. Since it will have only one kind of "atom" present, it will have certain characteristics not duplicated by any other element. What is an "atom"? One of the common elements is hydrogen which is a clear colourless gas, and is lighter than air. If it were possible to separate the smallest particle we could get, that would still have the characteristics of hydrogen, this tiny particle would be an atom. We might see something like this if it were possible to put it under a super-powerful microscope:



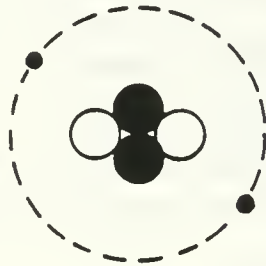
Hydrogen atom

The large black spot in the centre is the nucleus. In this case, the nucleus contains only one thing - a proton. It is a tiny particle that always has a positive charge.

The small black spot is a satellite, and the dotted circle represents its orbit. This satellite is called an electron. It is also a tiny particle but has a negative charge.

Since there is only one proton, we can say that hydrogen has a mass of 1. The electron, being so much smaller, is ignored as far as this mass is concerned.

Hydrogen is the lightest atom. Let us look at helium to show you why:

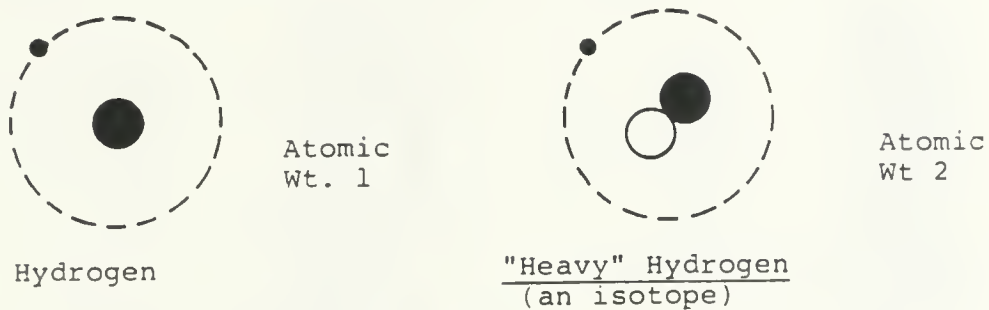


Helium Atom

In the helium atom nucleus, the two black spots represent two protons. The orbit has two electrons. The two small circles in the nucleus represent something new, two neutrons. A neutron is a particle which has the same weight as a proton but has no electrical charge (neutral). If we say that hydrogen has a mass of 1, then helium will have a mass of 4. This relative mass is called the atomic weight of an element. If we proceed down the list of elements, we will discover that each one has a different atom with a different arrangement of protons, neutrons and electrons. Using the number of protons and neutrons present, we can make a list of theoretical atomic weights.

Every once in a while we will find an odd example of an element mixed in with its sisters. It will have a slightly different arrangement of the nucleus, such as an extra neutron. This atom will be exactly like the others

except for weight and is called an isotope. An isotope might be described as a mutation of the usual atom.



If we could take a quantity of hydrogen atoms and weigh them, we would find that the average weight is not 1 but 1.008 due to the occasional presence of the heavier isotope. This explains why atomic weights are not all even numbers.

If you will turn to the accompanying table cataloguing the distribution of electrons in each orbit around the nucleus (Table 1-2), you will see that each orbit has only so many "parking spots" and once these are occupied, a new orbit shell is begun. An exception to this occurs in some of the outermost orbits where some "double parking" is overlooked. *In any case, there are never more than 8 electrons in the outer-most shell.*

On the basis of the distribution of electrons we can isolate four different structural types of atoms:

1. Inert elements - these with all orbit shells filled to the maximum (these are underlined in Table 1-2).
2. Simple elements - those with only one unfilled shell.

# Distribution of Electrons

At. No.	Element	Shells					At. No.	Element	Shells						
		k	l	m	n	o			k	l	m	n	o	p	q
1	Hydrogen	1					52	Tellurium	2	8	18	18	6		
2	Helium	2					53	Iodine	2	8	18	18	7		
3	Lithium	2	1				54	Xenon	2	8	18	18	8		
4	Beryllium	2	2				55	Cesium	2	8	18	18	8	1	
5	Boron	2	3				56	Barium	2	8	18	18	8	2	
6	Carbon	2	4				57	Lanthanum	2	8	18	18	9	2	
7	Nitrogen	2	5				58	Cerium	2	8	18	20	8	2	
8	Oxygen	2	6				59	Praseodymium	2	8	18	21	8	2	
9	Fluorine	2	7				60	Neodymium	2	8	18	22	8	2	
10	Neon	2	8				61	Promethium	2	8	18	23	8	2	
11	Sodium	2	8	1			62	Samarium	2	8	18	24	8	2	
12	Magnesium	2	8	2			63	Europium	2	8	18	25	8	2	
13	Aluminum	2	8	3			64	Gadolinium	2	8	18	25	9	2	
14	Silicon	2	8	4			65	Terbium	2	8	18	27	8	2	
15	Phosphorus	2	8	5			66	Dysprosium	2	8	18	28	8	2	
16	Sulfur	2	8	6			67	Holmium	2	8	18	29	8	2	
17	Chlorine	2	8	7			68	Erbium	2	8	18	30	8	2	
18	Argon	2	8	8			69	Thulium	2	8	18	31	8	2	
19	Potassium	2	8	8	1		70	Ytterbium	2	8	18	32	8	2	
20	Calcium	2	8	8	2		71	Lutetium	2	8	18	32	9	2	
21	Scandium	2	8	9	2		72	Hafnium	2	8	18	32	10	2	
22	Titanium	2	8	10	2		73	Tantalum	2	8	18	32	11	2	
23	Vanadium	2	8	11	2		74	Tungsten	2	8	18	32	12	2	
24	Chromium	2	8	13	1		75	Rhenium	2	8	18	32	13	2	
25	Manganese	2	8	13	2		76	Osmium	2	8	18	32	14	2	
26	Iron	2	8	14	2		77	Iridium	2	8	18	32	17	0	
27	Cobalt	2	8	15	2		78	Platinum	2	8	18	32	17	1	
28	Nickel	2	8	16	2		79	Gold	2	8	18	32	18	1	
29	Copper	2	8	18	1		80	Mercury	2	8	18	32	18	2	
30	Zinc	2	8	18	2		81	Thallium	2	8	18	32	18	3	
31	Gallium	2	8	18	3		82	Lead	2	8	18	32	18	4	
32	Germanium	2	8	18	4		83	Bismuth	2	8	18	32	18	5	
33	Arsenic	2	8	18	5		84	Polonium	2	8	18	32	18	6	
34	Selenium	2	8	18	6		85	Astatine	2	8	18	32	18	7	
35	Bromine	2	8	18	7		86	Radon	2	8	18	32	18	8	
36	Krypton	2	8	18	8		87	Francium	2	8	18	32	18	8	1
37	Rubidium	2	8	18	8	1	88	Radium	2	8	18	32	18	8	2
38	Strontium	2	8	18	8	2	89	Actinium	2	8	18	32	18	9	2
39	Yttrium	2	8	18	9	2	90	Thorium	2	8	18	32	18	10	2
40	Zirconium	2	8	18	10	2	91	Protactinium	2	8	18	32	20	9	2
41	Niobium	2	8	18	12	1	92	Uranium	2	8	18	32	21	9	2
42	Molybdenum	2	8	18	13	1	93	Neptunium	2	8	18	32	22	9	2
43	Technetium	2	8	18	14	1	94	Plutonium	2	8	18	32	23	9	2
44	Ruthenium	2	8	18	15	1	95	Americium	2	8	18	32	24	9	2
45	Rhodium	2	8	18	16	1	96	Curium	2	8	18	32	25	9	2
46	Palladium	2	8	18	18	0	97	Berkelium	2	8	18	32	26	9	2
47	Silver	2	8	18	18	1	98	Californium	2	8	18	32	27	9	2
48	Cadmium	2	8	18	18	2	99	Einsteinium	2	8	18	32	28	9	2
49	Indium	2	8	18	18	3	100	Fermium	2	8	18	32	29	9	2
50	Tin	2	8	18	18	4	101	Mendelevium	2	8	18	32	30	9	2
51	Antimony	2	8	18	18	5	102	Nobelium	2	8	18	32	31	9	2

TABLE 1-2

3. Transition elements - those with two unfilled shells.
4. Rare earth elements - those with three unfilled shells.

You will notice that the elements are arranged in the table in order by their atomic number. This is nothing more than the sum of the number of electrons in the shells surrounding the nucleus. Each element differs from its immediate neighbours by one electron and therefore one proton in the nucleus (since all elements must be electrically neutral).

### IONS

Some atoms, under certain circumstances can lose electrons and others can gain electrons. When an atom loses an electron, it will have an excess proton or one plus (+) charge and will be very active. Gaining an electron from another element causes an atom to become negatively (-) charged and also very active. This process is called ionization. Ions (electrically charged atoms) are formed. Ions are of two types depending on their charge:

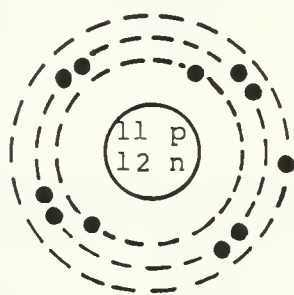
1. Anions - negatively charged ions - e.g.  $\text{Cl}^-$ ,  $\text{I}^-$ ,  $\text{Br}^-$
2. Cations - positively charged ions - e.g.  $\text{H}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$

### COMPOUNDS

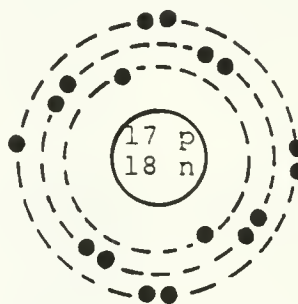
As we have said, compounds are pure substances made up of atoms of more than one element. Atoms of two or more elements combine to form a molecule of a compound with distinctive properties of its own. A molecule is the smallest particle of a compound which could be separated, that

would still show all of the properties of that compound. A molecule is usually made up of atoms of more than one element and is therefore somewhat larger in size than an atom.

When atoms combine to form molecules, only the outer shells of electrons take part in the joining. The nuclei are not affected. let us look at atoms of sodium and chlorine to see what makes them eager to join together to form a molecule of the compound sodium chloride (or table salt).



Sodium

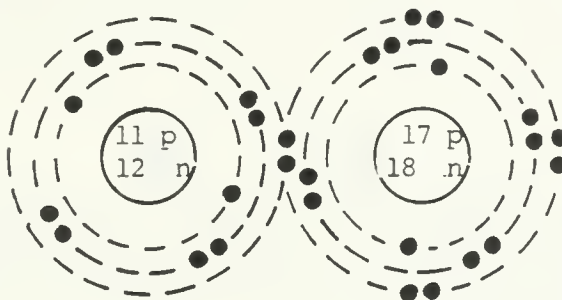


Chlorine

We can see that both atoms have completed inner electron shells with the maximum number of electrons. Neither has a complete outer orbit however. Chlorine has 7 electrons and is seeking one more electron to make a complete shell. This makes chlorine a very active element.

Sodium on the other hand has only one electron in its outer shell. It would gladly give up this electron to any atom that has a strong desire for it. This also makes sodium an active element.

If we could get the sodium atom and the chlorine atom to come together and make the transfer of a single electron, both would be happy. When these two elements contact each other, that is exactly what happens; two violently reactive elements combine to form a new, extremely stable compound.



SODIUM CHLORIDE

(table salt)

A few other examples of compounds formed this way are:  $\text{HCl}$ ,  $\text{KI}$ ,  $\text{H}_2\text{S}$  and  $\text{HNO}_3$ . With these last two,  $\text{H}_2\text{S}$  and  $\text{HNO}_3$ , you will notice that it is possible for several atoms to get into this exchanging act, if all of them will benefit by becoming more stable electrically.

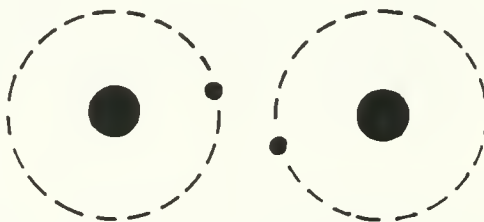
In nature, what we find is that elements that have highly reactive atoms, exist in their uncombined form

only as long as it takes to find another atom that can be convinced to undertake an electron exchange with it. When they find another compound already formed with a weaker element having those desirable atoms, this highly reactive element can force the weaker element to change places with it. In this sense, justice doesn't exist in the chemical world.

### MOLECULES

We have learned that atoms of two different elements can combine to form a molecule of a new compound, but a molecule can also be formed from atoms of the same element in some cases.

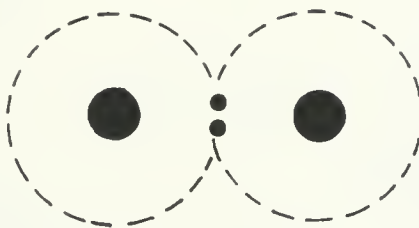
Certain elements, particularly those which are gases commonly combine with themselves to form molecules. Examples of this are:  $H_2$ ,  $O_2$  and  $Cl_2$ . The subscript "2" indicates that two atoms have formed a molecule. Let us look at hydrogen to explain this.



Hydrogen

Hydrogen

If these two atoms join together, they can share these two electrons and each will then have a stable outer shell of 2 electrons.



(Hydrogen Gas)

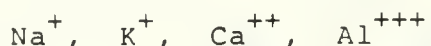
## VALENCE

The tendency of elements to form compounds through a shift of electronic structures is known as valence. Let us examine two methods of attaining a stable electronic distribution.

### A. Electrovalence

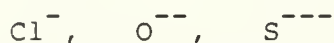
As we said earlier, under some conditions, an atom can lose one or more electrons, which leaves the atom with a corresponding number of tiny positive electrical charges. Other atoms can gain one or more electrons in a similar manner which will give them negative charges.

These positive and negative charges are equal and are attracted to each other by electrostatic action. Such atoms are said to be electrovalent and the term valence is used to describe the number of such bonds. Usually the ionized atoms of metals have positive charges (positive valence) and the symbols could be written as follows:



(Cations)

On the other hand, the atoms of nonmetals tend to become negatively charged or have negative valence. For example:



Electrovalent compounds are thus formed when anions and cations combine in the correct number to satisfy their valences. See Table 1-3, Table of Electrovalence

#### B. Covalence

On the basis of electrovalence we would expect an element like carbon to be fairly inert and form few compounds; yet, this element forms more compounds than all the other elements put together. Obviously, there must be some other valence mechanism.

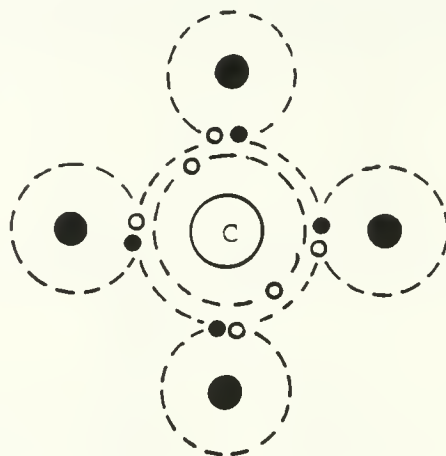
Carbon has four electrons in its outermost shell. Hydrogen has one electron in its only shell. If four hydrogen atoms were to approach a carbon atom so closely, that the shell of each hydrogen atom penetrated into the outmost shell of the carbon atom, the electrons in these interpenetrated shells would then be influenced by the nuclei of both types of atoms. Both atoms could then

TABLE 1-3

TABLE OF ELECTROVALENCE

POSITIVE (+) (CATIONS)	MONOVALENT (1)						BIVALENT (2)						TRIVALENT (3)						TETRAVALENT (4)						PENTRAVALENT (5)						HEXAVALENT (6)					
	H K Na Ag Hg Cu Au (NH <sub>4</sub> ) <sup>+</sup> • Ammonium • Radical						Ba Ca Co Mg Pb Zn Hg Cu Fe Mn Sn Cd						Al Au As Cr Fe P Sb Bi						C Si Mn Sn S						As P Sb Bi						Cr S					
NEGATIVE (-) (RADICALS)	F Cl Br I						O S						N P						C																	
	(OH) (HCO <sub>3</sub> ) (NO <sub>2</sub> ) (NO <sub>3</sub> ) (ClO) (ClO <sub>2</sub> ) (ClO <sub>3</sub> ) (ClO <sub>4</sub> ) (C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> ) (MnO <sub>4</sub> ) (HSO <sub>4</sub> )						(CO <sub>3</sub> ) (SO <sub>3</sub> ) (SO <sub>4</sub> ) (B <sub>4</sub> O <sub>7</sub> ) (SiO <sub>3</sub> ) (CrO <sub>4</sub> ) (C <sub>2</sub> O <sub>4</sub> )						(BO <sub>3</sub> ) (PO <sub>4</sub> ) (PO <sub>3</sub> ) (Fe(CN) <sub>6</sub> )						(Fe(CN) <sub>6</sub> )																	
	Hydrogen Potassium Sodium Silver Mercury (ous) Copper (ous) Gold (aurous) • Ammonium • Radical						Barium Calcium Cobalt Magnesium Lead Zinc Mercury (ic) Copper (cupric) Iron (ferrous) Manganese (ous) Tin (stannous) Cadmium						Aluminum Gold (auric) Arsenic (ous) Chromium Iron (ferric) Phosphorus (ous) Antimony (ous) Bismuth (ous)						Carbon Silicon Manganese (ic) Tin (stannic) Sulphur						Arsenic (ic) Phosphorus (ic) Antimony (ic) Bismuth (ic)						Chromium Sulfur					

share these electrons. What we would have is a carbon atom sharing one electron with each of four hydrogen atoms. In effect, the hydrogen electron would be spending part of its time orbiting the hydrogen nucleus and part of its time orbiting the carbon nucleus.



Covalent Bonding

Methane

In methane, each hydrogen atom now has two electrons, giving it the stable helium configuration in its orbits, and eight electrons are now associated with the carbon atom giving it the stable neon configuration. Both types of atoms have benefitted by attaining a stable structure through this sharing process. Covalent compounds are thus formed by the sharing of pairs of electrons.

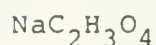
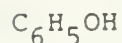
When only one pair of electrons is shared by the same two atoms, the bond is said to be a single bond. When two pairs are shared, we describe this as a double bond and with three pairs, a triple bond.

#### ORGANIC COMPOUNDS

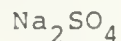
Most compounds which follow the covalent method of bonding just described and contain the element carbon, are, or have been at some time, part of the earth's life process. These are said to be organic compounds.

Compounds which do not fit into this category are labelled inorganic. For example:

Organic Compounds



Inorganic Compounds

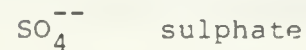
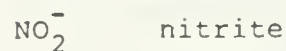
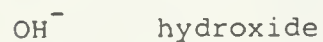
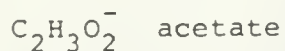


RADICALS

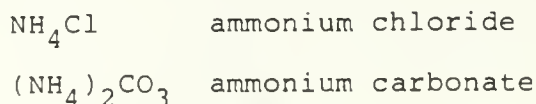
In many chemical compounds there are clusters of elements which behave as if they were a single element; such a group of elements is known as a radical.

Radicals exhibit some of the characteristics of ions, in that they all have an excess or a deficiency of electrons, causing the radical to possess an electrical charge. They will therefore combine with other ions or radicals to form compounds.

Common examples of radicals are:



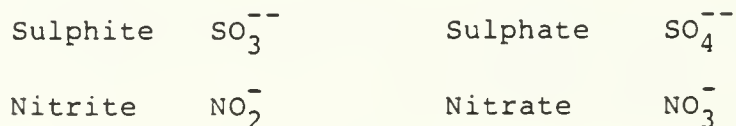
Since ammonium is a positive radical, it will form compounds with all negative ions or radicals. For example:



Note that it takes two ammonium radicals to satisfy the electrical charge (valence) of the carbonate radical.

If you look carefully at the names and formulas of the radicals you will notice that the suffixes "-ite" and "-ate" occur repeatedly. These suffixes are used only with radicals containing oxygen atoms. Notice that "-ite" radicals always contains fewer oxygen atoms than "-ate" radicals.

For example:



### MOLECULAR WEIGHT

When we were discussing atoms, we said that the relative mass of one atom compared to another is known as its atomic weight. We also said that the elements relative mass must be the average of its mass, taking into account the abundance of natural isotopes found.

Table 1-3 is a list of International Relative Atomic Weights scaled to the relative atomic mass of carbon as 12.

# International Atomic Weights

Name	Symbol	Atomic Weight	Name	Symbol	Atomic Weight	Name	Symbol	Atomic Weight
Actinium	Ac	227	Gold	Au	196.967	Potassium	K	39.102
Aluminum	Al	26.9815	Hafnium	Hf	178.49	Praseodymium	Pr	140.907
Americium	Am	243	Helium	He	4.0026	Promethium	Pm	147
Antimony	Sb	121.75	Holmium	Ho	164.930	Protactinium	Pa	231
Argon	Ar	39.948	Hydrogen	H	1.00797	Radium	Ra	226
Arsenic	As	74.9216	Indium	In	114.82	Radon	Rn	222
Astatine	At	210	Iodine	I	126.9044	Rhenium	Re	186.2
Barium	Ba	137.34	Iridium	Ir	192.2	Rhodium	Rh	102.905
Berkelium	Bk	249	Iron	Fe	55.847	Rubidium	Rb	85.47
Beryllium	Be	9.0122	Krypton	Kr	83.80	Ruthenium	Ru	101.07
Bismuth	Bi	208.980	Lanthanum	La	138.91	Samarium	Sm	150.35
Boron	B	10.811	Lawrencium	Lw	257	Scandium	Sc	44.956
Bromine	Br	79.909	Lead	Pb	207.19	Selenium	Se	78.96
Cadmium	Cd	112.40	Lithium	Li	6.939	Silicon	Si	28.086
Calcium	Ca	40.08	Lutetium	Lu	174.97	Silver	Ag	107.868
Californium	Cf	251	Magnesium	Mg	24.312	Sodium	Na	22.9898
Carbon	C	12.01115	Manganese	Mn	54.9380	Strontium	Sr	87.62
Cerium	Ce	140.12	Mendelevium	Md	256	Sulfur	S	32.064
Cesium	Cs	132.905	Mercury	Hg	200.59	Tantalum	Ta	180.948
Chlorine	Cl	35.453	Molybdenum	Mo	95.94	Technetium	Tc	99
Chromium	Cr	51.996	Neodymium	Nd	144.24	Tellurium	Te	127.60
Cobalt	Co	58.9332	Neon	Ne	20.183	Terbium	Tb	158.924
Copper	Cu	63.546	Neptunium	Np	237	Thallium	Tl	204.37
Curium	Cm	247	Nickel	Ni	58.71	Thorium	Th	232.038
Dysprosium	Dy	162.50	Niobium	Nb	92.906	Thulium	Tm	168.934
Einsteinium	Es	254	Nitrogen	N	14.0067	Tin	Sn	118.69
Erbium	Er	167.26	Nobelium	No	253	Titanium	Ti	47.90
Europium	Eu	151.96	Osmium	Os	190.2	Tungsten	W	183.85
Fermium	Fm	253	Oxygen	O	15.9994	Uranium	U	238.03
Fluorine	F	18.9984	Palladium	Pd	106.4	Vanadium	V	50.942
Francium	Fr	223	Phosphorus	P	30.9738	Xenon	Xe	131.30
Gadolinium	Gd	157.25	Platinum	Pt	195.09	Ytterbium	Yb	173.04
Gallium	Ga	69.72	Plutonium	Pu	242	Yttrium	Y	88.905
Germanium	Ge	72.59	Polonium	Po	210	Zinc	Zn	65.37
						Zirconium	Zr	91.22

TABLE 1-4

To find the relative mass of any molecule or its molecular weight, one simply adds up the atomic weights of each of the atoms in the molecule. For example:

1. HOCl (Hypochlorous Acid)

$$\begin{aligned}\text{Molecular weight} &= \text{H} + \text{O} + \text{Cl} \\ &= 1.008 + 15.999 + 35.453 \\ &= 52.460\end{aligned}$$

2. FeSO<sub>4</sub> (Ferrous Sulphate)

$$\begin{aligned}\text{Molecular weight} &= \text{Fe} + \text{S} + 4 (\text{O}) \\ &= 55.847 + 32.064 + 4 (15.999) \\ &= 151.907\end{aligned}$$

3. Ca(OH)<sub>2</sub> (Hydrated Lime)

$$\begin{aligned}\text{Molecular weight} &= \text{Ca} + 2 (\text{O} + \text{H}) \\ &= 40.08 + 2 (15.999 + 1.008) \\ &= 74.094\end{aligned}$$

Since these weights are only relative masses, they have no units. However, the proportions of each atom which combine to form a molecule are always the same. For example, HCl, hydrochloric acid will always contain 1.008 parts by weight of hydrogen and 35.453 parts by weight of chlorine. If the parts or units we choose are grams, HCl will always contain 1.008 grams hydrogen and 35.453 grams of chlorine in every 36.461 grams of HCl. In this way we can express the relative masses of atoms as gram atomic weights and their combined weights as gram molecular weights. These terms will become especially important to us later when we discuss the preparation of standard solutions.

## NAMING COMPOUNDS

### Binary Compounds

Binary compounds are those which are made up of two elements; simple examples are the salts NaCl and KCl. The names of these compounds consist of the names of the two elements, the positive element first, with the ending of the second element changed to "ide" -eg-

NaCl	-	sodium chloride
KCl	-	potassium chloride

If the metal has two different oxidation numbers (valences) this is indicated by the use of the suffix "-ous" for the lower or weaker example and "-ic" for the highest or stronger one -eg-

FeCl <sub>2</sub>	-	ferrous chloride
FeCl <sub>3</sub>	-	ferric chloride

Occasionally two elements can form into two or more compounds of different proportion and a different naming system is resorted to. The name of the second element is preceded by a prefix -eg- mono-(one), di-(two), tri-(three), tetra(four) etc. Oxides are good examples of this -eg-

Co	-	carbon monoxide
CO <sub>2</sub>	-	carbon dioxide
P <sub>2</sub> O <sub>3</sub>	-	phosphorous trioxide
P <sub>2</sub> O <sub>5</sub>	-	phosphorous pentoxide

### Compounds Containing Radicals

Naming compounds containing radicals is done in the same way as for binary compounds, except that the name for the radical is used rather than the names

of its component elements - eg -

$\text{CaCO}_3$	-	calcium carbonate
$\text{Na}_2\text{SO}_4$	-	sodium sulfate
$\text{NH}_4\text{Cl}$	-	ammonium chloride
$\text{KOH}$	-	potassium hydroxide
$\text{Fe}(\text{OH})_2$	-	ferrous hydroxide
$\text{Fe}(\text{OH})_3$	-	ferric hydroxide

### Acids

Binary acids are named using the prefix "hydro-" in front of the name of the negative element, followed by the suffix "-ic" -eg-

$\text{HF}$	-	hydrofluoric acid
$\text{HCl}$	-	hydrochloric acid
$\text{HBr}$	-	hydrobromic acid
$\text{HI}$	-	hydroiodic acid

Many common acid molecules contain hydrogen, a nonmetal (negative element), and oxygen. Since the amount of oxygen often varies, the name of the most common form of the acid in the series consists of the stem of the name of the nonmetal with the suffix "-ic". The acid containing one less atom of oxygen than the common form has the suffix "-ous". The acid containing one more atom of oxygen than the common form has the prefix "per-" and the suffix "-ic". The acid containing two less atoms of oxygen than the common form has the prefix "hypo-" and the suffix "-ous". -eg-

$\text{HNO}_3$	-	nitric acid
$\text{HNO}_2$	-	nitrous acid
$\text{HClO}_3$	-	chloric acid

$\text{HClO}_2$	-	chlorous acid
$\text{HClO}$	-	hypochlorous acid
$\text{H}_2\text{SO}_4$	-	sulfuric acid
$\text{H}_2\text{SO}_3$	-	sulfurous acid
$\text{H}_3\text{PO}_4$	-	phosphoric acid
$\text{H}_3\text{PO}_3$	-	phosphorous acid
$\text{H}_2\text{CO}_3$	-	carbonic acid
$\text{H}_2\text{C}_2\text{O}_4$	-	oxalic acid
$\text{H}_3\text{BO}_3$	-	boric acid



SUBJECT:

TOPIC: 2

SOLUTION PREPARATION

**OBJECTIVES:**

The student will be able to:

1. Select the correct definition from a given list for each of the following items:
  - a. solute
  - b. solvent
  - c. normality
  - d. molarity
  - e. percentage composition
2. Calculate the weight in grams of a given compound required to make a litre of 3 normal solution.
3. List the quantities represented in an indicated list of S.I. prefixes, by a given quantity in grams



## SOLUTIONS

A solution consists of two components, a solvent which is the dissolving medium and a solute which is the substance dissolved. The solute is dispersed as molecules or ions and the distribution of the solute is perfectly homogenous throughout the solution. Common examples of solvent and solute are:

SOLVENT	SOLUTE
water	sugar
alcohol	table salt
chloroform	baking soda
vinegar	starch

A concentrated solution is one which contains a relatively large amount of solute per unit volume of solution. A dilute solution is one which contains a relatively small amount of solute per unit volume of solution. The words "strong" and "weak" should not be used when referring to the concentration of a solution. Strong and weak are terms that are more properly used to describe the chemical activity of a substance.

## CONCENTRATION

The concentration of a solution can be expressed in a number of ways. The units of expression give an indication of the way in which a solution of this concentration would be made up. The most common units of expression for concentration are:

1. Molarity
2. Normality
3. Molality
4. Percentage Composition

## MOLARITY

The molarity of a solution is the number of gram molecular weights of solute per litre of solution. "Gram molecular weight" is sometimes abbreviated as mole, so molarity becomes

$$\text{Molarity (M)} = \frac{\text{Number of moles of solute}}{\text{Litre of solution}}$$

A solution which contains a half mole of solute per litre of solution would therefore be a 0.5 M solution.

## NORMALITY

The normality of a solution is the number of gram equivalent weights of solute per litre of solution. "Gram equivalent weight" is a new term and is often abbreviated as equivalent.

$$\text{Normality (N)} = \frac{\text{Number of equivalents of solute}}{\text{Litre of Solution}}$$

The equivalent weight of a compound is found from the net positive valence of the compound. If we take the valence of the positive (+) part of the compound and multiply it by its subscript we will have the total number of positive charges available, or net positive valence.

$$\begin{aligned} \text{net positive valence} &= (\text{valence of + element}) \\ &\quad \times (\text{its subscript if any}) \end{aligned}$$

The equivalent weight of any compound is then found from the relationship

$$\text{equivalent weight} = \frac{\text{molecular weight}}{\text{net positive valence}}$$

Let us use solutions of NaCl and  $K_2CO_3$  in water to illustrate this principle. To make up a 1 N solution of either salt we will need 1 gram equivalent weight (equivalent) of each dissolved in 1 litre of solution.

For NaCl, the net positive valence is 1 so the equivalent weight of NaCl is the same as its molecular weight.

For  $K_2CO_3$  however, the net positive valence is 2 so the equivalent weight of  $K_2CO_3$  is its molecular weight over 2.

#### MOLALITY

The molality of a solution is the number of moles of solute per 1000 grams of solvent. Since the solvent will not always be water, this method of expression is considerably different from molarity.

$$\text{molality (m)} = \frac{\text{Number of moles of solute}}{1000 \text{ grams of solvent}}$$

#### PERCENTAGE COMPOSITION

This method of expression of concentration may use either percentage by weight or percentage by volume as its units.

$$\% \text{ by weight} = \frac{\text{weight of solute}}{\text{weight of solution}} \times 100$$

$$\% \text{ by volume} = \frac{\text{volume of solute}}{\text{volume of solution}} \times 100$$

Percentage by weight is usually used in referring to solids dissolved in liquids. Percentage by volume is normally used with reference to gases in gases, or liquids in liquids.

#### CONCENTRATION EXAMPLE

If we were given the task of preparing a 3 N solution of  $K_2CO_3$ , we must start with the fact that

$$3N = \frac{3 \text{ equivalents of solute}}{\text{Litre of solution}}$$

What we now need to know is the gram equivalent weight of  $K_2CO_3$ . Let's start out by finding the gram molecular weight of  $K_2CO_3$ :

$$\begin{aligned} \text{gm. molecular wt } K_2CO_3 &= 2 (39.102) + 12.011 + 3 (15.999) \\ &= 138.212 \text{ gm} \end{aligned}$$

Since the valence of K is 1, the net positive valence of  $K_2CO_3$  is 2.

$$\begin{aligned} \text{gm equivalent wt of } K_2CO_3 &= \frac{\text{gm molecular wt}}{\text{net positive valence}} \\ &= \frac{138.212 \text{ gm}}{2} \\ &= 69.106 \text{ gm} \end{aligned}$$

Since, as we said earlier,

$$3N = \frac{3 \text{ equivalents of solute}}{\text{Litre of solution}}$$

we can now substitute

$$3N = \frac{3 (69.106 \text{ gm})}{\text{Litre of solution}}$$

$$= \frac{207.218 \text{ gm K}_2\text{CO}_3}{\text{Litre of solution}}$$

We can now make up this solution by weighing accurately 207.218 gm of dried  $\text{K}_2\text{CO}_3$ , placing this amount in a 1-litre volumetric flask and diluting the solution to occupy exactly 1 litre.

### SOLUTION PREPARATION

Where solutions of known concentration are used as standards in the analysis of other compounds, the preparation of these solutions must be undertaken with great care.

Atomic weights are given in the tables with great precision, and gram molecular or gram equivalent weights can be calculated to at least 4 decimal places. It is our responsibility then to see that only devices and procedures capable of continuing this precision are used in the preparation of standard solutions.

To prepare a standard solution, approximate desired quantities of granular or powdered solute are deposited in a weighing bottle, paper weigh-boat or aluminum weighing dish and dried for at least one hour in a laboratory oven at  $103^\circ \text{C}$ .

After drying, the weighing bottle containing the sample is placed in a laboratory desiccator to cool to room temperature in a dry atmosphere.



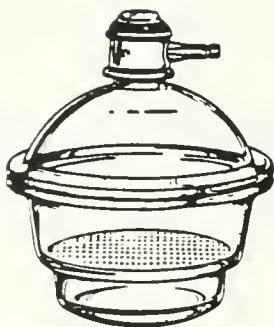
Weighing Bottle



Weigh-Boats



Weighing Dish



DESICCATOR



Single-pan Analytical Balance

The net weight of the solute is then determined using an analytical balance capable of 0.1 mg precision (0.0001 g). If any minor changes in the finished weight are desired, small amounts of solute may be removed at this time and the sample redried and cooled.

The sample is considered to have reached stable weight when three consecutive desicating and weighing cycles results in a difference of less than 1 mg. The mean of the three weight values is then taken as the weight of the sample.

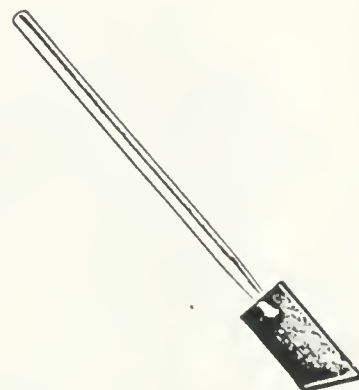
When the desired final weight is reached, the solute is transferred, using a powder funnel, from the weighing container to a 1 litre volumetric flask. A "rubber policeman" may be used to push the last few grains of powder into the funnel. A stream of distilled water from a wash bottle is then used to flush the weighing container and the powder funnel to dislodge and dissolve any stubborn powder.



Powder Funnel



Volumetric Flask



Rubber  
Policeman

The powder in the volumetric flask is dissolved in a small amount of distilled water by inverting or swirling the flask. When all of the powder is dissolved, enough distilled water is added to bring the level in the flask almost to the engraved line on the neck. The contents of the flask are then mixed by inverting several times.

When the solution is considered to be well mixed, it is allowed to stand for sometime so that all of the solution will drain back into the bottom of the flask. Then, using a fine-tipped wash bottle, distilled water is added slowly to bring the bottom of the meniscus curve exactly level with the engraved line.

After mixing the solution again by inversion at least twenty times, the solution must be standardized against some known laboratory standard solution to prove its concentration. The proven concentration of the solution is then marked clearly on the label of its container.

## THE S.I. (Metric) SYSTEM

The S.I. system of weights and measures is used in all laboratory work. The entire system is based upon the length of the meter, a bar of special metal which is carefully preserved in Paris. This bar represents one-tenmillionth of the distance from the equator to the North Pole. It serves as a standard for measuring distance, area and dry volume.

The unit of measure for weight is the gram. The units for weight, distance and liquid volume are related, in that the unit of measure for liquid volume is the litre, and 1/1000 litre (1 millilitre) of water at 39.2° F weighs one gram and occupies 1 cubic centimetre.

The system uses a variety of prefixes attached to these basic units in order to scale the units up or down conveniently. A list of the common prefixes appears in Table 2-1.

Prefixes Most Commonly Used with S.I.		
Prefix	Meaning	Symbol
mega-	$10^6$	M
kilo-	$10^3$	k
deci-	$10^{-1}$	d
milli-	$10^{-3}$	m
micro-	$10^{-6}$	u

TABLE 2-1

By our choice of prefix we can change very large numbers or very small numbers into convenient-sized readable numbers. For example:

36,000 metres	=	36 kilometres
0.0016 litres	=	1.6 millilitres
0.0000032 grams	=	3.2 micrograms

To change a number from one unit of the system to one with a different prefix, one simply moves the decimal point to the right or left the correct number of places. Using the prefixes in Table 2-1.

365 grams = 0.000365 megagrams  
          = 0.365 kilograms  
          = 3650 decigrams  
          = 365000 milligrams  
          = 365 000 000 micrograms



SUBJECT:

TOPIC: 3

LABORATORY STANDARD SOLUTIONS

OBJECTIVES:

The student will be able to:

1. Indicate whether a Primary Standard, Secondary Standard or Stock Solution should be used, given a list of required solutions.
2. Indicate which grade of chemical is required for indicated uses, given a list of standard chemical grades.
3. List three precautions for the correct handling of laboratory standards.
4. List three precautions for the correct storage of laboratory standards.



## STANDARD LABORATORY SOLUTIONS

In the laboratory, solutions of known concentrations are often used in the analysis of solutions of unknown concentrations. This procedure of comparing one solution to another is called Standardization. In the laboratory, several "levels" of standard solution are used to maintain the standards of accuracy expected. The commonly used laboratory standard solutions are:

1. Primary Standard
2. Secondary Standard
3. Stock Solution

### Primary Standards

Primary Standards are prepared solutions of known concentration which are purchased, accompanied by a certificate of analysis issued by the National Bureau of Standards, or its equivalent, for the purpose of standardizing analytical solutions. The concentrations of these solutions are known with great accuracy and all solutions in the lab are calibrated to this standard. In this way, all laboratories start out with the same opportunity to maintain accuracy.

A well-equipped laboratory will usually stock at least two primary standards; one for acid-base "neutralization" reactions and another for oxidation-reduction "redox" reactions.

Examples of common primary standard solutions are:

#### Neutralization

- a. acid potassium phthalate (potassium hydrogen phthalate)
- b. benzoic acid

## Redox

- a. sodium oxalate
- b. arsenic trioxide
- c. potassium dichromate

Great care must be taken to protect these solutions from contamination since all other lab solutions will be either directly or indirectly standardized against these solutions.

## Secondary Standards

It would not be practical for us to buy all of our lab solutions for quantitative analysis since the volume used would make the cost prohibitive. We therefore prepare the commonly-used solutions ourselves using analytical grade lab chemicals.

The solutions made up must then be calibrated against our Primary Standard with known concentration and known accuracy. Even though we use extreme care in preparing the solution from the finest grade of chemical available, the possibility of minute error always exists; so calibration is our only means of assuring ourselves that we have not introduced a deviation from true value into this "second generation" standard.

The calibration may be a direct one, such as the titration of a prepared 0.100 N solution of NaOH against a potassium acid phthalate Primary Standard. Or it may be an indirect calibration, such as the titration of a 0.100 N solution of  $\text{H}_2\text{SO}_4$  against the 0.100 N NaOH Secondary Solution just prepared. This indirectly calibrated solution now becomes a "third generation" standard and any measurement error we have created has been compounded.

Standardization beyond the third generation is to be discouraged since the possibility of error is greatly increased.

Most solutions used in the lab for measurement of the concentration of other chemicals (quantitative analysis) are of the Secondary Standard class. The accuracy of concentration of these solutions is a direct measure of the care and precision of the technician in their preparation and calibration.

Secondary Standard solutions must always be labelled clearly and stored carefully to prevent their contamination. The containers used for their storage must be scrupulously cleaned and rinsed with the standard solution before their use.

The solution in the bottle must never come in contact with any other item of glassware or apparatus. For use, the solution is poured into a pre-cleaned beaker or flask and measured out from this container. Left-over solution must never be returned to the standard solution; it must be discarded.

### Stock Solutions

Many solutions are used daily in the lab as part of the preparation of a sample for quantitative analysis. Large volumes of these solutions may be used and the accuracy of their concentration may not be so critical (since the solution does not take part in the actual analysis). In these situations, a stock solution may be adequate.

Stock solutions are often prepared in concentrated form as a Secondary Standard and then small quantities of this solution are diluted to make up the less-concentrated

working solution or stock solution. For example, a measured quantity of 10.000 N.  $\text{H}_2\text{SO}_4$  Secondary Standard solution may be diluted 100 times using volumetric glassware to give a 1/10 N (or N/10) solution for daily bench use.

The stock solution may also be made up directly by weighing out the required amount of dry chemical and dissolving this in the correct amount of solvent.

### Chemical Grades

Chemicals are produced and sold by lab suppliers in a number of different grades of purity. In general, the greater the purity of the chemical, the higher its price. Depending on the use for the chemical and the quantity used, we must choose the grade with the desired characteristics and price.

If we intended to use 150 pounds of the chemical to make up a cleaning solution for pipes, we would probably look for the cheapest source of this chemical, possibly an industrial grade.

On the other hand, if we were making up a Secondary Standard solution for quantitative analysis to the nearest mg/l, we would probably use the highest available analytical grade.

In general, the chemicals made specifically for lab use are classified in one of the following grades:

1. Ultra-Pure Grade

These products are produced by the finest technology available and are very expensive. Each container is accompanied by a certificate of lot analysis

listing the assay of the desired chemical itself and the quantities of any impurities present. These reagents are suitable for use in gas chromatography and spectrophotometry.

2. A.C.S. Grade

These products meet the specifications as listed in "Reagent Chemicals" published by the American Chemical Society. Certificates of lot analysis usually will accompany each container.

3. Analytical Grade

These products meet specifications set by the supplier only. Specifications usually define minimum standards of purity. Assays and principal constituents are expressed in terms of minimum values and impurities as maximum values.

4. U.S.P., F.C.C. and N.F.

These designations define chemicals that conform to the requirements of the current U.S. Pharmacopeia, Food Chemicals Codex and National Formulary respectively.

5. Purified Grade

This grade defines chemicals of good quality where there are no official standards.

6. Practical Grade

This grade designates those chemicals of sufficiently high quality to be suitable for use in many syntheses

and diverse applications. These chemicals may contain impurities that make them useful for only certain analytical applications.

## 8. Technical Grade

This grade designates some chemicals of selected commercial quality. Technical grade products are useful in some applications and, where applicable, are more economical.

### Uses for Lab Grades

For the preparation of standard solutions in the water or wastewater laboratory, only A.C.S. Grade chemicals should be used. Some Analytical Grade chemicals may meet or exceed A.C.S. specifications, but these are usually identified by their suppliers.

For the preparation of preservative solutions, cleaning solutions and some indicator solutions, lower grade reagents may be acceptable. The levels of specific impurities will decide whether the lower grade is acceptable or not. When in doubt, for lab purposes, use the higher grade.

### Handling of Lab Standards

As mentioned earlier, lab standard solutions must be handled with great care to prevent contamination which may result in a change in concentration. A few of the basic rules are as follows:

1. Never touch anything to the standard solution in the bottle, instead, pour out the required amount and reclose the container.

2. Never return any left-over amount of the standard solution to the container.
3. Never touch the stopper or cap from the standard container to any other surface.
4. If the solution is poured into a wet container, its concentration has changed.
5. Containers used for storage of standard solutions should be rinsed several times with small quantities of that solution before being filled for storage.
6. Never switch the stoppers or caps from bottle to bottle while they are containing standard solutions.

#### Storage of Lab Standards

The storage of lab standard solutions as well as their handling must be considered very carefully. A few basic rules, if followed carefully, will prevent most storage problems.

1. Standard solutions should be kept in a cool, dry, dark location.
2. Containers used for the storage of standard solutions should be made of resistant borosilicate glass such as Corning "Pyrex" or Kimble "Kimax". Special glassware is available with characteristics such as high resistance to alkali attack, low boron content or exclusion of light.

3. Stoppers and caps must be resistant to attack by the solution in the bottle.
4. Glass stoppers are unsatisfactory for strongly alkaline liquids because of their tendency to "freeze" or seize.
5. Some standard solutions such as sodium thio-sulphate require preservations to combat biological growth. Instructions for the preparation of the solution will specify any preservatives required.
6. Some standard solutions alter slowly because of chemical or biological changes. The practical life, required frequency of standardization or storage precautions are indicated for such standards.

In any case, do not consider a standard valid for more than one year unless it is restandardized.

SUBJECT:

TOPIC: 4

LABORATORY EQUIPMENT

**OBJECTIVES:**

The students will be able to:

1. Recognize and indicate the following pieces of laboratory equipment, given a collection of illustrations:
  - a. flask
    - i) Erlenmeyer
    - ii) Florence
    - iii) Filter
    - iv) Volumetric
  - b. beaker
  - c. pipet
    - i) Mohr
    - ii) Volumetric
  - d. buret
  - e. bench reagent bottle
  - f. BOD bottle
  - g. stock solution bottle
  - h. graduated cylinder
  - i. syringe
  - j. desiccator
  - k. separatory funnel
  - l. drying oven
  - m. muffle furnace
  - n. analytical balance
  - o. trip balance
2. List a use for each of the above items.
3. List the relative precision of each of the above items which is a measurement device.
4. Describe what is meant by "Class A Standard " when applied to the precision and accuracy of pipets, burets and volumetric flasks.



## BASIC LABORATORY EQUIPMENT

Since many water and wastewater treatment plant laboratories are being expanded to improve their ability to monitor the process, it is important that we know at least a few basic principles for the purchase of new equipment. Laboratory supply catalogues contain a bewildering array of glassware and equipment. It's not uncommon, for example, for a supply catalogue to list 80 different models of flask, each model available in several different sizes.

In order to narrow down the choices to those items acceptable for performance of the test, we should know a bit about terminology, materials and precision.

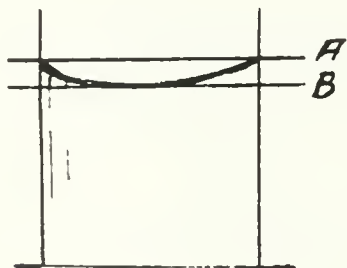
### Types of Glassware

Laboratory vessels serve three functions: (1) storage of reagents, (2) measurement of solution volumes, and (3) confinement of reactions. It may be advantageous for special purposes to use vessels made from materials such as porcelain, nickel, iron, aluminum, platinum, stainless steel and plastic. Glass, however, is the most widely used material of construction. There are many grades and types of glassware from which to choose ranging from student grade to others possessing specific properties; such as resistance to thermal shock, alkali, low boron content, and super strength. Soft glass containers are usually relatively soluble and, therefore, are not recommended for general use. They especially are not recommended for the storage of reagents. The mainstay of the modern analytical laboratory is a highly resistant borosilicate glass. This glass is manufactured by Corning Glass Works under the name "Pyrex" or by Kimble Glass Company as "Kimax".

The use of plastic vessels, containers and other apparatus made of Teflon, polyethylene, polystyrene and polypropylene has increased markedly over recent years. Some of these materials, such as Teflon, are quite expensive; however Teflon stopcock plugs have practically replaced glass plugs in burets, separatory funnels, et cetera because lubrication to avoid sticking or "freezing" is not required. Polypropylene, a methylpentene polymer, is available as laboratory bottles, graduates, beakers and even volumetric flasks. It is crystal clear, shatter-proof, autoclavable, and chemically resistant.

### Volumetric Analyses

By common usage, accurately calibrated glassware for precise measurements of volume has become known as volumetric glassware. This group includes volumetric flasks, volumetric pipets and accurately calibrated burets. Less accurate types of glassware including graduated cylinders, serological and measuring pipets also have specific uses in the analytical laboratory when exact volumes are unnecessary.



Reading of Meniscus  
Correct reading is  
along line B.

The precision of volumetric work depends in part upon the accuracy with which volumes of solutions can be measured, and there are certain sources of error which must be carefully considered. The volumetric apparatus must be read carefully; that is, the bottom of the meniscus should

be tangent to the calibration mark. However, there are other sources of error; such as changes in temperature which result in changes in the actual capacity of the glass apparatus, in the volume of the glass apparatus, and in the volume of the solutions.

The capacity of an ordinary glass flask of 1,000 ml volume increases 0.025 ml per degree rise in temperature. However, if the flask is made of borosilicate glass, the increase is much less. One thousand millilitres of water or of most 0.1 N solutions increase in volume by approximately 0.20 ml per 1 degree of Celsius increase at room temperature. Thus, solutions must be measured at the temperature at which the apparatus was calibrated. This temperature (usually 20 degrees Celsius) will be indicated on all volumetric ware. They may also be errors of calibration of the apparatus; that is, the volume marked on the apparatus may not be the true volume. Such errors can be eliminated only by recalibrating the apparatus or by replacing it.

Volumetric apparatus is calibrated "to contain" or to "deliver" a definite volume of liquid. This will be indicated on the apparatus with the letters "TC" (to contain) or "TD" (to deliver). Volumetric flasks are calibrated to contain a given volume. They are available in various shapes and sizes ranging from 1,000 to 2,000-ml capacity.

Volumetric pipets are calibrated to deliver a fixed volume. The usual capacities are 1 through 100 ml although micropipets are also available. In emptying volumetric pipets, they should be held in a vertical position and the outflow should be unrestricted. The tip of the pipet is kept in contact with the wall of the receiving vessel for

a second or two after the free flow has stopped. The liquid remaining in the tip is not removed; this is most important.

Measuring and serological pipets should also be held in a vertical position for dispensing liquids; however, the tip of the pipet is only touched to the wet surface of the receiving vessel after the outflow has ceased. For those pipets where the small amount of liquid remaining in the tip is to be blown out and added, indication is made by a frosted band near the top.

Burets are used to deliver definite volumes. The more common types are usually of 25- or 50-ml capacity, graduated to tenths of a millilitre and provided with stopcocks. For precise analytical methods in microchemistry, microburets are also used. Microburets generally are of 5- or 10-ml capacity, and graduated in hundredths of a millilitre division. Automatic burets with reservoirs are also available ranging in capacity from 10 to 100 ml. Reservoir capacity ranges from 100 to 4,000 ml.

General rules in regard to the manipulation of a buret are as follows. Do not attempt to dry a buret which has been cleaned for use, but rinse it two or three times with a small volume of the solution with which it is to be filled. Do not allow alkaline solutions to stand in a buret, because the glass will be attacked and the stopcock unless made of Teflon will tend to freeze. A 50-ml buret should not be emptied faster than 0.7 ml per second; otherwise, too much liquid will adhere to the walls and as the solution drains down, the meniscus will gradually rise giving a high false reading. It should be emphasized that improper use of and/or reading of burets can result in serious calculation errors.

In the case of all apparatus for delivering liquids, the glass must be absolutely clean so the film of liquid never breaks at any point. Careful attention must be paid to this fact or the required amount of solution will not be delivered. The various cleaning agents and their uses are described later.

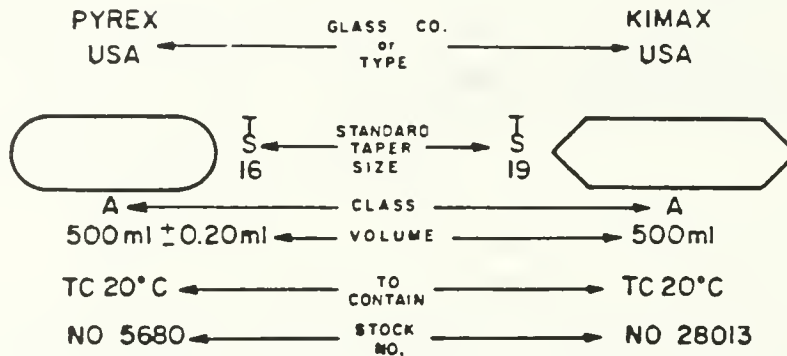
#### U.S. Federal Specifications for Volumetric Glassware

Circular 602 of the National Bureau of Standards, "Testing of Glass Volumetric Apparatus", describes the Federal specifications for volumetric glassware. The National Bureau of Standards no longer accepts stock quantities of volumetric apparatus from manufacturers or dealers for certification and return for future sale to consumers. This certification service is still available, however, apparatus will be tested only when submitted by the ultimate user, and then only after an agreement has been reached with the National Bureau of Standards concerning the work to be done.

Consequently, the various glass manufacturers have discontinued the listing of the National Bureau of Standards certified ware. In its place, catalog listings of volumetric glass apparatus which meet the Federal specifications are designated as Class A, and all such glassware is permanently marked with a large "A". The ware in question includes the usual burets, volumetric flasks, and volumetric pipets.

In addition to the "A" marking found on calibrated glassware and the temperature at which the calibration was made, other markings also appear. These include the type of glass; such as Pyrex, Corex, Kimax, et cetera; the

stock number of the particular item; and the capacity of the vessel. If the vessel contains a ground glass connection, this will also be included along with the "TC" symbol. An example of the markings usually found on volumetric ware is shown below.



EXAMPLE OF MARKINGS ON  
GLASSWARE

Class A glassware need not be recalibrated before use. However, if it should become necessary to calibrate a particular piece of glassware, directions may be found in texts on quantitative analysis.

#### Cleaning of Glass and Porcelain

The method of cleaning should be adapted to both the substances that are to be removed and the determination to be performed. Water soluble substances are simply washed out with hot or cold water, and the vessel is finally rinsed with successive small amounts of distilled water. Other substances more difficult to remove may require the

use of a detergent, organic solvent, dichromate cleaning solution, nitric acid or aqua regia (25 per cent volume to volume concentrated nitric acid in concentrated hydrochloric acid). In all cases, it is good practice to rinse a vessel with tap water as soon as possible after use. Material allowed to dry on glassware is much more difficult to remove.

Volumetric glassware, especially burets, may be thoroughly cleaned by a mixture containing the following: 30 g sodium hydroxide, 4 g sodium hexametaphosphate (trade name -Calgon), 8 g trisodium phosphate, and 1 litre water. A gram or 2 of sodium lauryl sulfate or other surfactant will improve its action in some cases. This solution should be used with a buret brush.

Dichromate cleaning solution (chromic acid) is a powerful cleaning agent; however, due to its destruction nature upon clothing and upon laboratory furniture, extreme care must be taken when using this mixture. If any of the solution is spilled, it must be cleaned up immediately. Chromic acid solution may be prepared in the laboratory by adding 1 litre of concentrated sulphuric acid slowly while stirring to 35 ml of saturated sodium dichromate solution. This mixture must be allowed to stand for approximately 15 minutes in the vessel which is being cleaned and may then be returned to a storage bottle. Following the chromic acid wash, the vessels are rinsed thoroughly with tap water and then with small successive portions of distilled water. A persistent greasy layer or spot may be removed by acetone or by allowing a warm solution of sodium hydroxide, about 1 g per 50 ml of water, to stand in the vessel for 10-15 minutes. After rinsing with water, dilute with hydrochloric acid and water again. Then the vessel is usually clean. Alcoholic potassium hydroxide is also effective in removing grease. To dry glass apparatus, rinse with acetone and blow or draw air through it.

### Special Cleaning Requirements

Absorption cells used in spectrophotometers should be kept scrupulously clean, and free of scratches, fingerprints, smudges and evaporated film residues. The cells may be cleaned with detergent solutions for removal of organic residues, but they should not be soaked for prolonged periods in caustic solutions because of the possibility of etching. Organic solvents may be used to rinse cells in which organic materials have been used. Nitric acid rinses are permissible, but dichromate is not. Rinsing and drying of cells with alcohol or acetone before storage is a preferred practice. Matched cells should be checked to see that they are equivalent by placing portions of the same solution in both cells and taking several readings of the transmittance (% T) or optical density (OD) values. If a cell is mismatched, it should be discarded or reserved for rough work.

Glassware to be used for phosphate determinations should not be washed with detergents containing phosphates. This glassware must be thoroughly rinsed with tap water and distilled water. For ammonia and Kjeldahl nitrogen, the glassware must be rinsed with ammonia free water.

### WEIGHING EQUIPMENT

Accurate measurement of the weight of chemicals for making up reagents and for solids measurements is critical for good analysis. The instrument used for weighing in the laboratory is called a "balance". A balance is defined as an instrument for determining the mass (weight) of a body by comparison with a series of other bodies whose masses (weights) are known.

There are two types of balances commonly used in the laboratory. One is used to weigh in the range of one gram

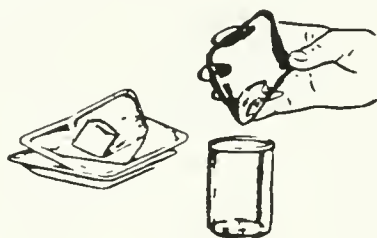
to one kilogram with an accuracy of 0.1 gram. This is referred to as a "trip" balance and is adequate for many routine tests.

Whenever the weight to be measured is less than five grams, a more precise instrument called an "analytical" balance is used. This balance has a range usually going from 0.001 to 200 grams with an accuracy of 0.1 milligram. This instrument is very delicate and must be used with care. It must rest on a solid surface that is not subject to vibration, heat or draft. There are two kinds of analytical balances. These are the double pan balance and the single pan balance. The double pan balance has a "pan" hanging on each end of a beam that is supported in the exact center on a knife edge. Weights are placed on one pan, and the material being weighed is placed in the other pan. An indicator points to a zero point when both pans and contents are of equal weight. For the accuracy needed only Class S weights should be used. The weights must be handled only by the use of the forceps provided for that purpose. Skin oils and dirt from handling with human fingers will add weight that is measurable when measuring the small amounts used with this balance.

The single pan balance is an instrument that has weights built in, and these weights are controlled mechanically by dials. Only one pan is provided, upon which the material being weighed is placed. This balance is the most widely used today as the weight is measured more quickly and simply with less chance of error.

Analytical balances are enclosed in glass with sliding doors to keep out dust and dirt which will affect accuracy and to keep out air movement while weighing. A small brush of fine hair is used to "sweep" out dust and material that may be spilled during weighing.

It can be difficult to remove the pan and materials weighed from the balance. To overcome this problem, a weighing paper or dish is placed on the pan to place the material to be weighed in or on. The paper or dish is weighed and compensated for in the final weight of the material being weighed. Disposable plastic weighing dishes in several sizes are available, and dry chemicals can be rinsed from them for greater accuracy.



Plastic Weighing Dishes

#### Lab Equipment Uses

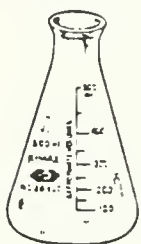
The following items are common laboratory apparatus and are used in practically all chemical analyses. Just as screwdriver or wrench is a generic term with many specialized types and uses, "beaker" and "pipet" are generic terms. The number of variations of the pipet for example is almost endless and each has its own specialized application. We can however, list the general areas of use of each of the indicated items.

<u>Equipment Item</u>		<u>Uses</u>
1. Flask	a) Erlenmeyer, Florence	- reaction container - coarse liquid volume measurement
	b) Filter	- collection of filtrate - application of vacuum to filter medium
	c) Volumetric	- precise liquid volume measurement
2. Beaker		- reaction container - coarse liquid volume measurement
3. Pipet	a) Volumetric	- precise liquid volume measurement (to contain)
	b) Mohr	- delivery of small measured quantities of liquid in odd amounts
4. Buret		- delivery of precise amounts of liquid
5. Bench Reagent Bottle		- handy storage of small amounts of frequently-used reagents -eg - $H_2SO_4$ , $HCl$ , $NaOH$ , $NH_4OH$ , $HNO_3$ - etc -
6. Stock Solution Bottle		- storage of larger amounts of frequently used chemicals, especially prepared solutions.
7. B.O.D. Bottle		- test for wastewater strength.
8. Graduated Cylinder		- less precise liquid volume measurement.
9. Syringe		- delivery of small, measured amounts of viscous solutions such as polymers
10) Dessicator		- a dry atmosphere for sample cooling
11) Separatory Funnel		- separation of two layers of immiscible liquids - eg - oil and water
12) Drying Oven		- drying of lab samples to constant weight
13) Muffle Furnace		- controlled burning of organic samples to ash
14) Analytical Balance		- precise weight measurement
15) Trip Balance		- less precise weight measurement.

TABLE 4-1I

## COMMON EQUIPMENT ITEMS

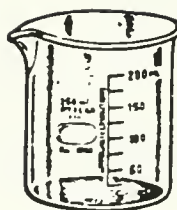
### A. Containers



Erlenmeyer  
Flask



Florence  
Flask



Beaker



Bench Reagent  
Bottles



Stock Solution  
Bottles

Figure 4-1

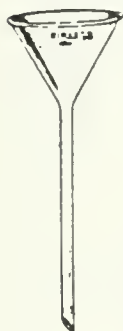


B.O.D. Bottle

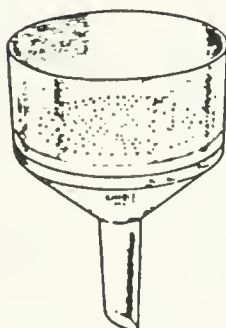


Separatory Funnel

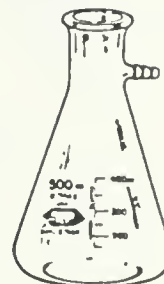
## B. Filtering Apparatus



Filter Funnel



Buchner Funnel



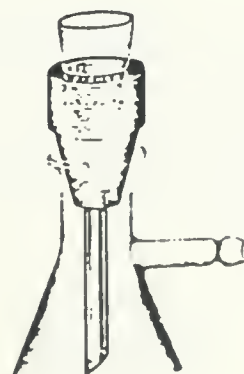
Filter Flask



Glass

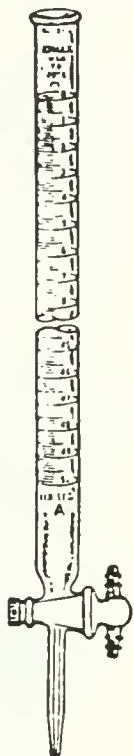


Porcelain  
Cooch Crucible



Walter Filter  
Holder

C. Volumetric Glassware



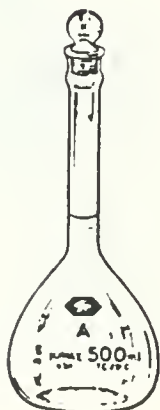
Buret



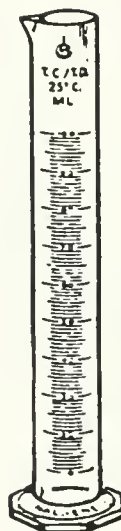
Volumetric  
Pipets



Mohr

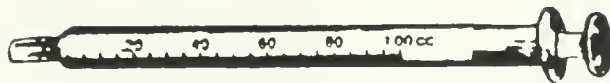


Volumetric Flask



Graduated Cylinder

Figure 4-3



Syringe (for viscous liquids)

D. Gravimetric Apparatus



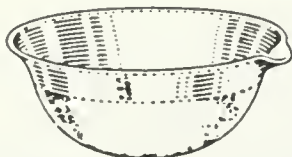
Crucible



Crucible tongs



Weighing Bottle



Evaporating Dish

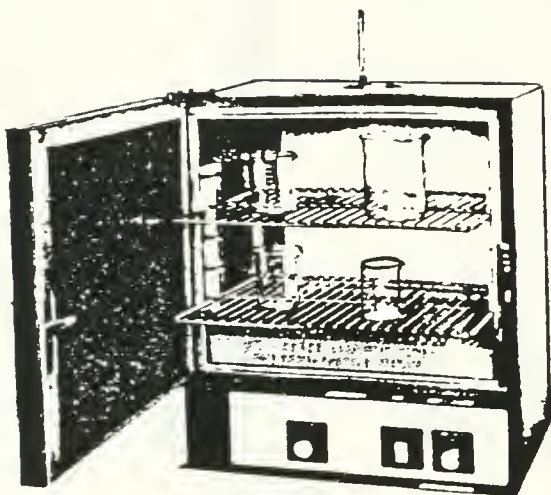


Powder Funnel

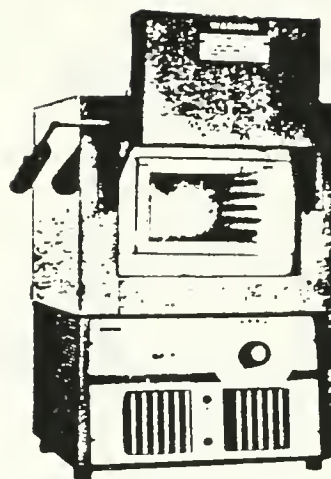


Desiccator

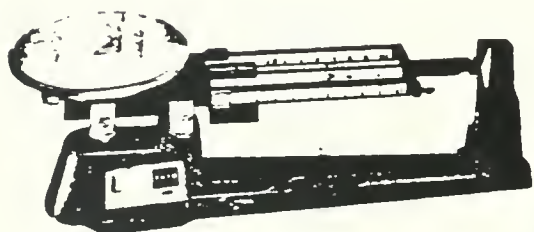
Figure 4-4



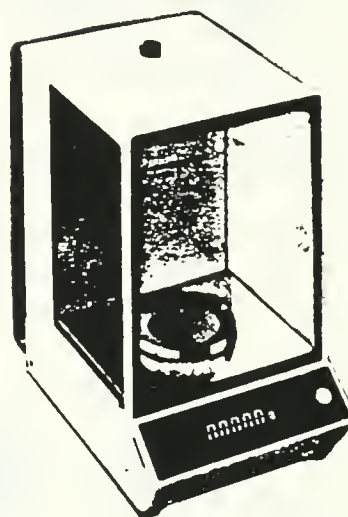
Drying Oven



Muffle Furnace



Trip Balance



Analytical Balance

Figure 4-5

## Measurement Precision

Whether we are considering weight measurement or volume measurement we are faced with a variety of equipment with a variety of precisions. Some items are meant for coarse measurement of large amounts, others for precise measurement of small amounts. It is important for the lab operator to understand the reason for this variety.

No-one would suggest, for example, that we measure the water for our coffee by the bucketful. Or on the other hand, no-one would suggest we measure the gas into our car by the cupful. The precision called for in these cases is obvious. Laboratory practice can be just as obvious.

The instructions given for preparation of a solution will indicate to you what precision is required. For example, an instruction to weigh out 56 gm of solute would immediately indicate that the trip balance is acceptable for this job. An instruction to weigh out 13.465 gm on the other hand, calls for the use of an analytical balance.

The relative precision of familiar measurement items is listed in Table 4-2.

<u>Measurement Item</u>	<u>Precision</u>
beaker, flask	+ 5%
graduated cylinder	+ 0.5%
volumetric flask	+ 0.30 ml in 1 litre + 0.02 ml in 10 millilitre
volumetric pipet	+ 0.08 ml in 100 ml + 0.05 ml in 50 ml + 0.006 ml in 1 ml
buret	+ 0.10 ml in 100 ml + 0.05 ml in 50 ml
Mohr pipet	+ 0.16 ml in 50 ml + 0.01 ml in 1 ml
Trip balance	+ 0.1 gm
Analytical balance	+ 0.0001 gm

TABLE 4-2

Only those burets emptying through a nozzle permanently attached at the bottom are accepted for NBS testing.

Side tubes, unless provided with stopcocks, are not permitted on burets.

So-called Schellbach burets - that is, those having a milkglass background with a coloured centre line - will not be accepted for testing because of possible errors due to parallax.

The distance between the extreme graduations must not exceed 70 cm on burets.

The rate of outflow of burets must be restricted by the size of the tip. For the graduated length the time of free outflow must not be more than 3 minutes nor less than the following for the respective lengths.

National Bureau of Standards  
Specification of Burets

Length Graduated, cm	Time of out- flow not less than, sec.	Length Graduated, cm	Time of out- flow not less than, sec
15	30	45	80
20	35	50	90
25	40	55	105
30	50	60	120
35	60	65	140
40	70	70	160

On 50 ml and 100 ml burets, the highest graduation mark should be not less than 4 cm nor more than 10 cm from the upper end of the buret. On burets having a capacity of 25 ml or less, this distance should not be less than 3 cm nor more than 6 cm. (These requirements do not apply to burets where "zero" is at end of an overflow tip).

Buret tips should be made with a gradual taper of from 2 cm to 3 cm, the taper at the extreme end being slight.

A sudden contraction at the orifice is not permitted, and the end of the tip must be ground perpendicular to axis of tube. The outside edge should be beveled slightly and all ground surfaces polished.

In order to facilitate the removal of drops and to avoid splashing, tip may be bent slightly.

In filling burets, excess liquid adhering to the tip should be removed when completing the filling.

#### The Measure of a Pipet

The true measure of a pipet is clearly defined in specifications established and outlined by The National Bureau of Standards and various other agencies. In order to meet their standards, certain criteria must be followed in the calibrating process.

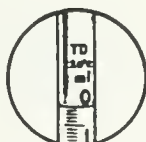
- Temperature - Calibrating temperature is 20<sup>o</sup> C. Should pipets be used at other temperatures, variations are usually so slight as to be negligible.
- Delivery Time - Minimum and maximum delivery times have been established for the various sizes and types of pipets. The size of the pipet tip regulates the rate of outflow; any alteration of delivery time may affect pipetting accuracy.
- Calibrating Fluids - To meet federal standards, pipets must be calibrated with either distilled water or mercury. "To deliver" pipets are always calibrated with distilled water. "To contain" pipets are always calibrated with mercury.

- To Contain (TC) - Pipets designated as "to contain" are calibrated by introducing into them the exact weight of mercury required to give the required volume. Mercury does not wet glass. Pipets calibrated with mercury will contain, but not deliver, the stated volume of aqueous fluid - a film of water will always cling to the wall of the pipet. "To contain" pipets must not be blown out.



TC - To Contain

- To Deliver (TD) - "To deliver" pipets are calibrated by weighing the volume of distilled water that will flow from them by gravity with the tip against the side of the receiving vessel. A small amount of liquid remains in the tip and must not be blown out.



TD - To Deliver

- To Deliver with Blown Out - Calibrated of "to deliver with blown out" pipets is similar to that used for "to deliver" pipets, except that the drop remaining in the tip after delivery is blown into the receiving vessel. These pipets are usually identified by a double etched band (frosted) at the top.



Double Etched Band  
(To Deliver with Blow-Out)

- Volumetric (Transfer) Pipets have a bulb midway between the mouthpiece and the top. The bulb decreases the surface area per unit volume and diminishes the possible error resulting from water film. Volumetric pipets should be used when a high degree of accuracy is required. Generally calibrated "to deliver". Volumetric and transfer pipets are one and the same.
- Oswald-Folin Pipets are volumetric pipets designed for use with viscous fluid. They are made from narrow bore tubing and have a bulb near the tip. They are ususally calibrated "to deliver with blow-out".
- Measuring (Mohr) Pipets are made from straight bore tubing and have multiple graduations. They are calibrated with water and therefore generally of the "to deliver" type. They are guaranteed accurate only at the maximum calibration mark. the accuracy of the fractional calibration marks depends on the uniformity of the pipet bore; the marks are made by dividing the length of the pipet containing the total volume into the required number of divisions by linear measurement. Measuring pipets are used when a high degree of accuracy is not essential. A graduated pipet having a maximum capacity near the volume required will give the greatest degree of accuracy (i.e. a 10.0 ml pipet used to measure 8.0 ml).
- Micropipets are considered to be those with capacity of 1 ml or less. They are usually "to contain" types.
- Serological Pipets have multiple graduations. They are "to deliver" or may be "to deliver with blow-out". Available sterile or non-sterile; plugged or not plugged; reusable or disposable.

SUBJECT:

TOPIC: 5

BASIC MASS MEASUREMENT

**OBJECTIVES:**

The student will be able to:

1. List the relative precision of the following mass measurement devices in terms of significant digit production.
  - a) Trip Balance
  - b) Analytical Balance
2. Indicate what mass measurement device is called for, given the instructions for preparation of a practice solution.
3. Select examples from a given list to indicate the following terms:
  - a) Accuracy
  - b) Sensitivity
  - c) Precision
  - d) Readability
  - e) Capacity



## MASS and WEIGHT

What fundamental property of matter do we measure when we weigh an object? Exactly what do we mean when we say, for example, that a man's weight is 180 lb? How can it be that this same man weighs only 30 lb when he stands on the surface of the moon? How can a man weigh zero at one point in his space journey when he weighed 900 lb. or more during lift-off? To answer these questions, we have to realize that there is a difference between mass and weight. Mass is the fundamental measure of the amount of a substance. Weight is a measure of the effect of gravitational force on that mass. When he stands on the earth's surface, the mass of the astronaut is pulled toward the center of the earth with a force of 180 lb. On the surface of the moon, the same man is pulled toward the center of the moon with only 1/6 as much force, and his weight is only 1/6 of 180 lb or 30 lb.

Although it is strictly accurate to speak of the mass of a sample when referring to the amount, most people call this property the weight of the sample. This is correct because the weighing is being done under the same gravitational conditions. The pull of gravity is so very nearly constant in laboratories everywhere on the earth that differences in the weight of the same mass can be detected only with very special instruments. Since the manipulations you will do are properly called weighings, our discussion will deal with your results, the weights of objects.

As we said in Topic 4, the two common weight-measurement devices used in the lab are the trip balance and the analytical balance. These two devices differ considerably in their precision (sensitivity). The trip balance is a coarse weight-measurement device capable of weighing to the

nearest 0.1 g. The analytical balance, on the other hand is a very precise device capable of weighing to at least the nearest 0.0001 g (0.1 mg).

### The Analytical Balance

It has only one pan and two knife edges. The weights are on the same side of the fulcrum as the pan. In order to obtain a "balance", side A must "weigh" the same as side B. In the case of the single-pan balance, the counter weight on side B plus the weights of the rest of the parts on that side of the knife edge are made equal to the weight of the pan, the weights, and the other parts on side A of the knife edge. Therefore, we have a "balance" with nothing on the pan. What happens when we put a sample on the pan? Suppose the sample weighs 10 g. By turning a knob on the front of the balance case, we remove 10 g of weight from the beam and the weights are in "balance" again. Figure 5-2 shows a balance of this type in more detail.

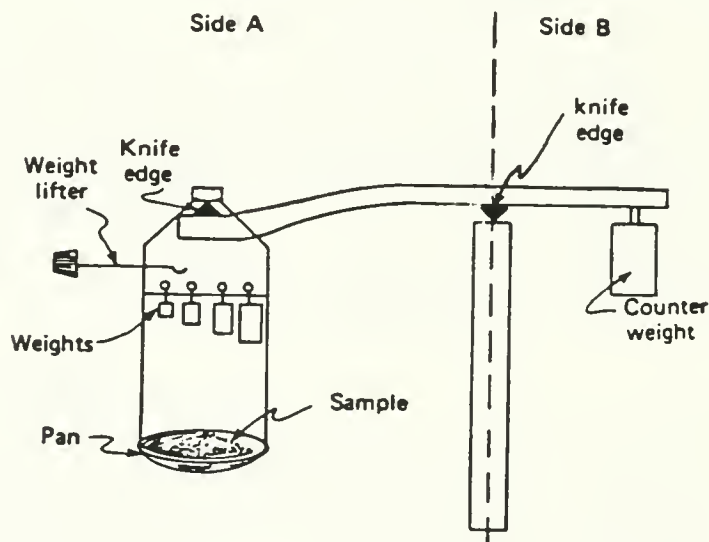


Figure 5-1  
The Single-Pan Balance Principle

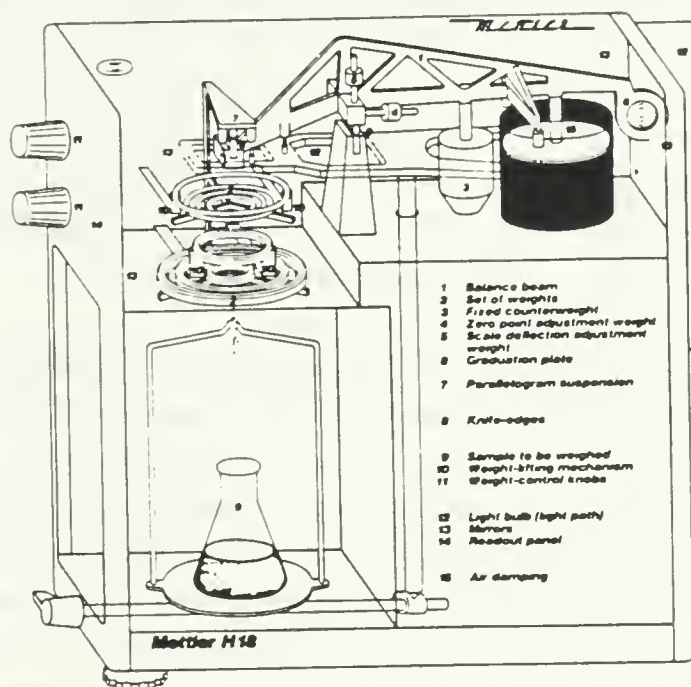


Figure 5-2  
 A Commercial Single-Pan Balance

When the sample is added to the pan, an equal weight (or combination of weights) is removed from the same side of the balance. This process is called weighing by substitution.

By removing weight equal to the weight of the sample, the total weight of side A is always the same regardless of how heavy the sample is. The weight on side A is always the same as that on side B. This constant total mass is important for accurate weighings because the sensitivity of the balance remains the same regardless of the weight of sample. Sensitivity is the smallest difference in weight that a balance can detect.

An analytical balance will measure differences in weight as small as one-tenth of a milligram. Just what does that mean? the following two weights differ by one-tenth of a milligram (0.0001 g): 6.2918 g and 6.2919 g. To make such accurate weighings requires good technique and some practice and, above all, a balance which has been handled carefully and maintained in good condition.

The word accuracy is defined as the "closeness to the true value". While we should always strive to be accurate in any measurement, we cannot afford the time it takes to obtain a higher degree of accuracy than is needed. It would make little sense to use an analytical balance for rough weighing of reagents when the exact amount is unimportant. Balances are available that measure with varying degrees of accuracy. Analytical balances can weigh to the 4th and 5th decimal places when weights are in grams. For example, a single-pan "4-place balance" can perform weighings such as  $5.2321 \pm 0.0001$  g and a "5-place balance" is capable of measuring weights' such as  $5.23211 \pm 0.00002$  g. Even more accurate balances are available which can weigh to 6, 7 and even 8 decimal places.

#### Balance Accuracy

What are the criteria used to determine which balance to choose for a specific weighing operation? The answer to that question depends on the answers to further questions. First, what is the accuracy required? Based on using convenient quantities of material, what balance is necessary? Let us look at a real situation.

Mr. D. Dobber has sampled the soil in his yard and has returned to the laboratory with 10 g of representative material. He would like to have 5 different constituents

measured. You are asked to analyze the material for the ash content - the material remaining after high-temperature burning. Your first decision is to determine the size of soil sample to use for this particular measurement. Since other tests must be done and you would like to keep some of the sample in reserve in case something goes wrong, you might choose to use 1 g. Let us assume that this soil contains 90.0% ash; this is, 0.9 g will remain after burning the 1 g sample. What will be the accuracy of the answers if you choose a balance which will weigh  $1 \text{ g} \pm 0.1 \text{ g}$ ? This means that the sample weight might vary from 0.9 g to 1.1 g ( $1 \pm 0.1 \text{ g}$ ). Now let us calculate the percent of ash by using the extremes. The highest sample weight is 1.1 g. The lowest ash weight is obtained by multiplying the lowest sample weight, 0.9 g, by the percent ash in the sample, 90% as shown;

$$\text{lowest ash weight} = 0.9 \text{ g (lowest sample weight)} \times 0.90 = 0.8 \text{ g}$$

Now, the percent ash can be calculated as shown:

$$\frac{0.8 \text{ g (lowest ash weight)}}{1.1 \text{ g (highest sample weight)}} \times 100 = 72.7\% \text{ ash}$$

Now let us calculate the percent ash using the lowest sample weight and the highest ash weight.

$$\frac{1.0 \text{ g (highest ash weight)}}{0.9 \text{ g (lowest sample weight)}} \times 100 = 111.1\%$$

Based on balance accuracy alone, the result could have varied from 72.7 to 111.1%. This, of course, would be intolerable.

Let us see what would happen if you used a balance which was accurate to  $\pm 0.01 \text{ g}$ . The initial 1 g sample could vary between 0.99 g and 1.01 g and the ash could vary from 0.89 to 0.91 g. Let's check the calculation:

$$\frac{0.89 \text{ g (lowest ash weight)}}{1.01 \text{ g (highest sample weight)}} \times 100 = 88.1\% \text{ ash}$$

$$\frac{0.91 \text{ g (highest ash weight)}}{0.99 \text{ g (lowest sample weight)}} \times 100 = 91.9\% \text{ ash}$$

Obviously, the variation is much less using this balance. This may or may not be good enough.

Let us go one step further and suggest that you use a balance which weighs to  $\pm 0.001$  g. The sample weight could vary from 0.999 g to 1.001 g and the ash could vary from 0.899 g to 0.901 g. Let's repeat our calculations:

$$\frac{0.899 \text{ g}}{1.001 \text{ g}} \times 100 = 89.8\% \text{ ash}$$

$$\frac{0.901 \text{ g}}{0.999 \text{ g}} \times 100 = 90.2\% \text{ ash}$$

With this balance the ash content is determined to be  $90 \pm 0.2\%$ . For accurate work this is the balance to be chosen. If you had only 0.1 g of soil available, a balance capable of measuring to  $\pm 0.0001$  g or better would be necessary for the same accuracy.

### Balance Precision

Another question in balance selection is "what is the precision of the balance? Precision differs from accuracy in that precision is a measure of the ability to reproduce a measurement. An official of the National Bureau of Standards describes the difference between precision and accuracy as: "Accuracy has to do with the closeness to the truth; precision only with the closeness together". For example, a highly precise balance may get out of adjustment and give inaccurate weighings. Suppose you have a weight certified by the National Bureau of Standards to be

1.0000 g and you weigh it three times to get values of 0.9854 g, 0.9853 g, 0.9855 g. Your balance has a precision of 0.00002 g but it is obviously inaccurate.

Other questions relating to the choice of a balance have to do with the readability and capacity. These are defined as:

Readability: The smallest fraction of a division which can be read with accuracy.

Capacity: The largest load that the balance can handle. Substitution balances are limited by the total amount of weights that can be removed.

### Handling and Care

We have described the degrees of accuracy and precision which can be obtained from a balance. This is achieved only when the balance is handled with care. We will now describe proper techniques for using this instrument.

There are a few basic rules for handling and caring for the balance:

1. Always raise the knife edges before you move a balance, place anything on, or remove any object from the pan.
2. Place the balance out of direct sunlight and away from drafts and corrosive liquids.
3. A balance should always be level.
4. Close the windows around the pan and replace the dust cover when the balance is not in use.

5. Keep the balance pan as clean as possible. Dirt, rust or oily fingerprints are easily weighed.  
CAUTION: Never leave spilled solids or liquids inside the weighing area or on the balance pan. use a small, soft brush or tissue paper and remove this material immediately.
6. Never force the controls of a balance. If something is stuck, call the instructor.
7. Always return the balance settings to zero when weighing is finished.
8. Never place samples directly on the pan; instead use a weighing bottle, dish, weighing pan, watch glass or piece of weighing paper. Several of these are shown in Figure 5-3.

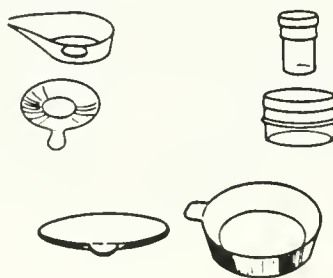


Figure 5-3  
Weighing Dishes and Pans

Some balances provide for subtracting the weight of the empty container. This is called taring and is easily done by turning an adjustment knob which sets the balance to read zero when the empty container is in the pan.

For highest accuracy, weighing bottles should not be touched with the fingers. The oil from fingerprints

is easily weighed and may collect dust, which causes an even greater error. Weighing bottles can be handled with a folded piece of paper as shown in Figure 5-4.

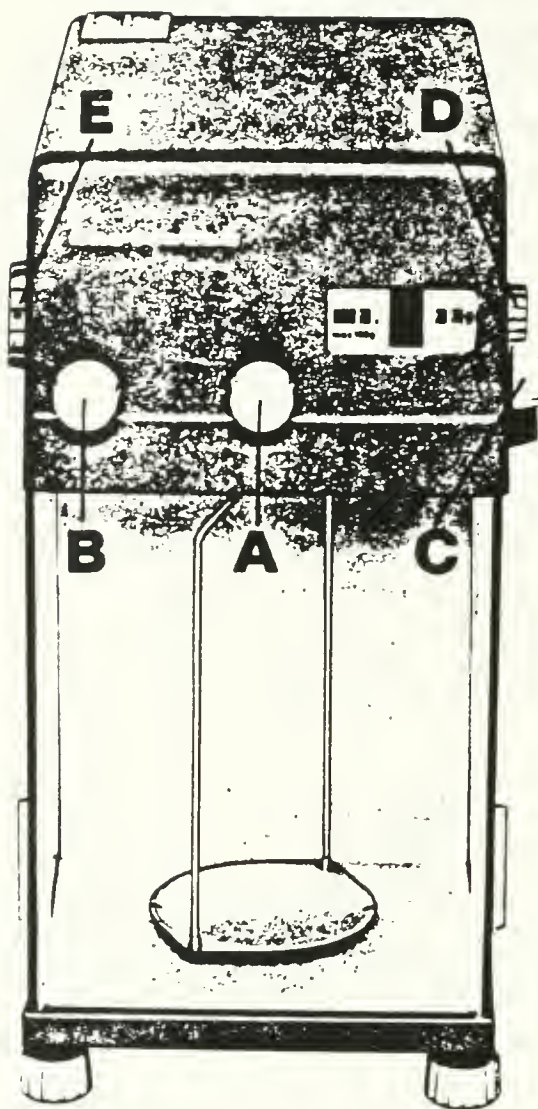
The paper strips are made from a 2 inch wide scrap of paper folded lengthwise. Tongs and forceps are used to handle containers other than weighing bottles.



Figure 5-4  
Handling Weighing Bottles

#### Weighing Procedure

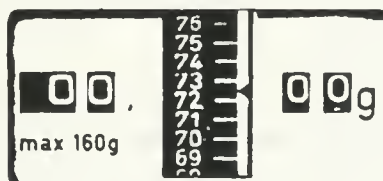
1. Before weighing be sure that the balance is level. The bubble in the spirit level should be centred within the indicator. If not, adjust the foot screws of the balance until the bubble is centered.
2. With the pan empty and balance doors closed, swing the arrest knob to the "free" position. When the optical scale stops moving, turn the zero adjustment knob until the zero line of the optical scale is perfectly aligned with the index mark. Arrest the balance by swinging the arrest knob to the "rest" position. The zero point should be rechecked each time the balance is used.



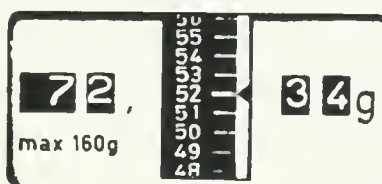
- A - Knob for dial weights 1-9 g
- B - Knob for dial weights 10-150 g
- C - Micrometer knob (fine setting)
- D - Zero point adjustment knobs
- E - Arrest knob

Figure 5-5  
Balance Controls

3. Place sample on balance pan and turn release lever up to position, "pre-weighing". The coarse weight to the nearest gram is now indicated on optical scale. Read the number below the index mark. Weight indication: 72 g.



4. Dial weight knobs to this number.
5. Fully release balance beam. After the optical scale has come to a standstill, turn the micrometer knob until the reading mark coincides with one line of the topical scale. Example: weight indication: 72,5234 g. Arrest the balance. Record weight.



### Factors Effecting Accuracy

#### Temperature

Several factors influence the accuracy of an analytical balance including temperature, static charge and buoyancy.

If an object on a balance pan has a temperature different from the surrounding air, a weighing error will result. Consider the following case: a porcelain crucible has been ignited in a muffle furnace and not cooled completely.

Warm air above the crucible will expand and become less dense. Cooler air will push up from below the balance pan causing "lift".

A  $1^{\circ}\text{C}$  difference in temperature will change the indicated weight of a crucible by about 0.2 mg. This is not acceptable for analytical work. As a general rule store materials to be weighed for at least 30 minutes in a desiccator and place the desiccator next to the balance 15 minutes before a weighing is made.

How do you tell if the object is not at the same temperature as the air in the balance? Put the object on the balance pan and weigh it. Wait 10-15 seconds and see if the object still weighs the same. A slow change in indicated weight usually means a temperature difference. Of course, if you take 10-15 minutes to make a weighing, the drifting may be due to moisture being absorbed. A weighing should never take more than one minute when using a single-pan balance.

### Static Electricity

Static charges become important only when the humidity is less than 45%. If you wipe a flask, beaker, crucible, etc., with a dry cloth before you weigh it, this may produce a static charge on the vessel. Also, silk and nylon clothing may produce static charges which can be transferred to the vessels. Therefore, it is good technique not to wipe vessels to be weighed; static charge may produce as much as a 20 mg error in the weighing. A vessel with a static

charge will cause the balance to become erratic, making it impossible to read.

Objects made of quartz are the worst offenders. Those made of hard glass also cause problems while objects made of soft glass are difficult to charge. For this reason weighing bottles are made of soft glass.

Some ways to remove a static charge are (1) touch the vessel to a water pipe (which grounds it and removes the static charge), (2) hold it under an ultraviolet lamp, (3) set it next to a low-level source of radiation or (4) simply let it stand for about 15 minutes in a desiccator.

### Buoyancy

If you have ever had the experience of retrieving a solid object such as a stone from the bottom of a swimming pool, you recognize that an object feels much lighter in water than it does in air. This is because you feel (you are measuring) the weight of the object relative to the water in which it lies. We say that a buoyant force is acting on the object. This force is equal to the weight of the water displaced by the object. If the density (weight/volume) of an object is less than the density of the water, the buoyant force is greater than the pull of gravity and it must be held under the surface. If it is released it will float on the surface. (An object goes down into a liquid until it displaces a weight of water equal to the total weight of the object; if it goes under the surface of the water before this happens, it sinks).

When we weigh an object in air, we must realize that it is actually displacing a volume of air just as a stone at the bottom of a swimming pool has displaced some of the

water. The same kind of buoyant force exists. The difference is that whereas the displaced water weighs a lot, the displaced air weighs very little. However, if you are using a very sensitive balance, this amount of buoyancy is significant (1 litre of air weighs about 1 gram). As a result, a big, bulky, low-density object that appears to weigh 15.0000 g and a small, more dense object that also appears to weigh 15.0000 g do not actually weigh the same. The big object is buoyed up by the air more than the small one and therefore appears to weigh a little less than it actually weighs. The effect of buoyancy can amount to 1 or 2 mg for objects such as weighing bottles, but this is easily corrected by weighing samples by difference.

#### The Desiccator

A desiccator is a container which is used to dry and store samples in a low moisture atmosphere. It consists of four parts:

1. The body in which a drying agent (desiccant) is placed.
2. A ground glass lid which slides onto the body to form an airtight seal.
3. A plate on which to place articles to be dried or maintained at low moisture levels.
4. The desiccant, a material which can absorb large amounts of water. It should also be easily regenerated by heating and be inexpensive.

## Use of the Desiccator

1. The ground glass rim of the desiccator should be coated with a thin film of grease to make an airtight seal.
2. The body is filled with desiccant to within 3/4 inch of the plate.  $\text{CaCl}_2$  is inexpensive, but it should be renewed as soon as it begins to appear "caked". Indicating Drierite ( $\text{CaSO}_4$ ) is another useful desiccant and has the added advantage of turning from blue to pink as it absorbs moisture.
3. Any object taken from a flame or furnace should be permitted to cool for at least two minutes before it is placed into a desiccator. A general rule is that if heat can no longer be felt on the back of your hand when it is held 2 inches from the object, then the object can be put in a desiccator. If hot objects are placed inside a desiccator, a vacuum forms upon cooling, making it difficult to remove the lid. If a vacuum forms, the desiccator must be opened very slowly or the air rushing into the desiccator may blow the samples out of the crucibles.
4. A desiccator should not be left open longer than is absolutely necessary. Moisture from the air will saturate the desiccant prematurely.
5. When opening a desiccator, slide the lid sideways; never try to lift it off.
6. Carry the desiccator with one hand around the front (like a football) so that one hand is free for another operation, such as opening a door.



SAMPLING

**OBJECTIVES:**

The student will be able to:

1. Define   a) Grab Sample  
             b) Composite Sample
2. Indicate, given a drawing of plant processes, the commonly accepted sampling locations in a complete water treatment and a complete Conventional Activated Sludge plant.
3. Given the correct designation for a sample taken from each of the above locations.
4. List the correct sample preservation techniques for three given analyses.



## SAMPLING

### PURPOSE

The purpose of routine sampling is to obtain data concerning the physical, chemical and biological characteristics of the process flow regardless of the size or type of plant. This information can be used for control of the treatment processes, to show that regulations or standards have been observed, and for design of plant extensions.

### TYPES OF SAMPLES

#### Grab Sample

A grab sample can be defined as a single sample of water taken without considering the time or the rate of flow. It is not very useful for process calculations since a single aliquot is not usually representative of average conditions. Grab samples are only of value in determining the composition of either maximum or minimum flows.

Some analyses must be performed immediately as preserving the sample would change the analytical results. In these instances, the grab sample is the only method we can use. Examples of analyses that must be determined on grab samples are dissolved oxygen, temperature, odour, oxygen uptake rate, and chlorine residual.

#### Composite Sample

A composite sample is defined as one which is built up, or composited from a series of grab samples taken at intervals during a fixed sampling period. It represents the average characteristics of the plant flow over the survey period and may be used for process calculations using the flow volume over the same period.

Composite samples are of two types:

1. Constant volume
2. Flow-proportional

In the first type, a constant volume is taken at each interval regardless of the flow rate at the time. If the flow rate is not constant, the flow rate should be recorded with each sample. usually these samples are then analyzed separately and their results averaged to represent the characteristics of the flow. Multiplying each sample result by a factor to account for the flow rate at sampling time would then give us an even more representative value for the average.

In the second type of composite, the amount of the aliquot taken at each interval must change in proportion to the flow rate at the time when the sample is withdrawn from the process stream. For example, if at one sampling time the measured flow rate is 150,000 gpd and an aliquot of 500 ml is taken, at the next sampling only 250 ml should be taken if the measured flow rate is 75,000 gpd. All samples are then combined for analysis.

With either type of composite sample, a constant time interval is usually chosen between aliquots, since most variations in waste characteristics occur on a time cycle. The time allowed between aliquots is determined by the variability of process stream characteristics. If the characteristics vary rapidly, the aliquots must be taken frequently, say every half hour, while if the waste is of fairly uniform character, the intervals may be longer, say every hour. The most accurate average will, of course, be given by a continuously drawn sample, but unless automatic

equipment is used, this will not be practical. Therefore, longer intervals, such as 10, 15 or 30 minutes are often used. However, aliquots should never be taken less than once per hour. A similar principle applies when characteristics vary with volume.

Alternately for a flow-proportional composite, the sample volume may remain the same and the interval between samples taken may be varied according to flow. Essentially this results in more samples being taken during high flow periods, thus weighting the average in proportion to the volume of flow. This principle is most often used in automatic sampling where a motor drive or clock timer is driven at a speed proportional to a varying flow signal.

#### TYPES OF SAMPLING DEVICES

There are two types of sampling devices, automatic and manual. Examples of each with their advantages and limitations are as follows:

1. Automatic

- a) Vacuum Sampler

This apparatus consists of an evacuated sample container, an electrically (or mechanically) operated closing device and a length of tubing. The open end of the tube is placed in the flow stream and the timing mechanism is set to operate at the required intervals. When the closing device is opened, wastewater is drawn into the container.

Advantages of this apparatus include simplicity, reasonable costs and ability to function for long periods on a small storage battery. Its chief limitation is that it can only be used on a minimum lift.

b) Pump Sampler

This apparatus consists of a sample container, a pump, and interconnecting tubing. Many types of pumps may be used, among the most useful being chemical feed pumps, due to their ability to meter accurately small volumes of liquid. A timing mechanism may be incorporated.

Both vacuum and pump samplers may be equipped to sample in proportion to the flow rate.

c) Blow-out Sampler

This apparatus consists of a sample collector in the flow stream, a source of compressed air and interconnecting tubing. The sample collector is maintained in the "normally-open" position. A timing mechanism causes pressurizing of the sample collector and at the same time, valving off of the entrance to the sample collector. This results in the contained air pressure "blowing out" the sample to the sample jug. Once the pressure is released, the sample collector returns to its normally-open condition, allowing another sample aliquot to enter.

- d) In addition to the two types described above, there are many others based on similar principles and on various types of wheels, discs, dippers and rotating scoops.

## COLLECTION OF SAMPLES

In keeping with the prime objective of a sampling programme (to obtain accurately representative samples), certain precautions must be taken to avoid errors. Whatever type of container is used, it must, of course, be "clean"; rinse it with sample to be taken before collecting the sample. Sterile "bacti" sample bottles must not be rinsed before use.

NOTE: Where smaller samples are to be taken from a larger sample, care should be taken that the sample is representative (shake or stir the sample well to ensure a thoroughly mixed smaller sample).

## SAFETY IN SAMPLING

No sample is worth obtaining at the risk of life and limb. Safety precautions should be observed at all times, including the following:

1. Never sample alone at night where lighting is poor,
2. Never enter a confined space, tank or other vessel or a sewer unless it cannot be avoided, and then only when it is known that the atmosphere contains sufficient oxygen and is free of explosive, toxic or noxious gases and there is no possibility of any material entering while sampling is in progress.

Never enter without outside assistance and always use a lifeline.

3. Always remember that most organic solvents are highly flammable and form explosive mixtures with air. Smoke only in safe places and when sampling is completed.

4. All chemical substances must be considered harmful (until proven otherwise) if ingested into the stomach or lungs, or by contact with the skin and eyes. It is vital to know what materials may be encountered during a survey and to use all necessary protection devices.

#### WHERE TO SAMPLE AND TYPE OF SAMPLE REQUIRED

REMEMBER: THE ANALYSIS IS ONLY AS GOOD AS THE SAMPLE TAKEN!

Taking good, representative samples of the water entering and leaving the treatment plant is extremely important. The type of sample, where it is taken and how it is taken require conscientious care and attention since they will greatly influence the reliability of the data obtained.

The following points must be considered when taking a sample as well as where the sample is taken:

##### A. Water Treatment

##### 1) (51) Raw Water

Grab samples of the plant influent prior to any chemical additions or treatment are a necessity in order to assess the treatment required. The frequency may vary from hourly to daily depending on the variation in the raw water quality.

##### 2) Filter Influent (Settled Water)

Grab samples at this point are necessary in order to assess the turbidity being applied to the filters. Frequency will again be dependent on the quality of the raw water.

- 51 Raw Water
- 52 Treated Water
- 58 Filter Backwash
- 59 Clarifier Sludge

# **WATER TREATMENT** **TYPICAL FILTRATION PLANT** SURFACE WATER SUPPLY

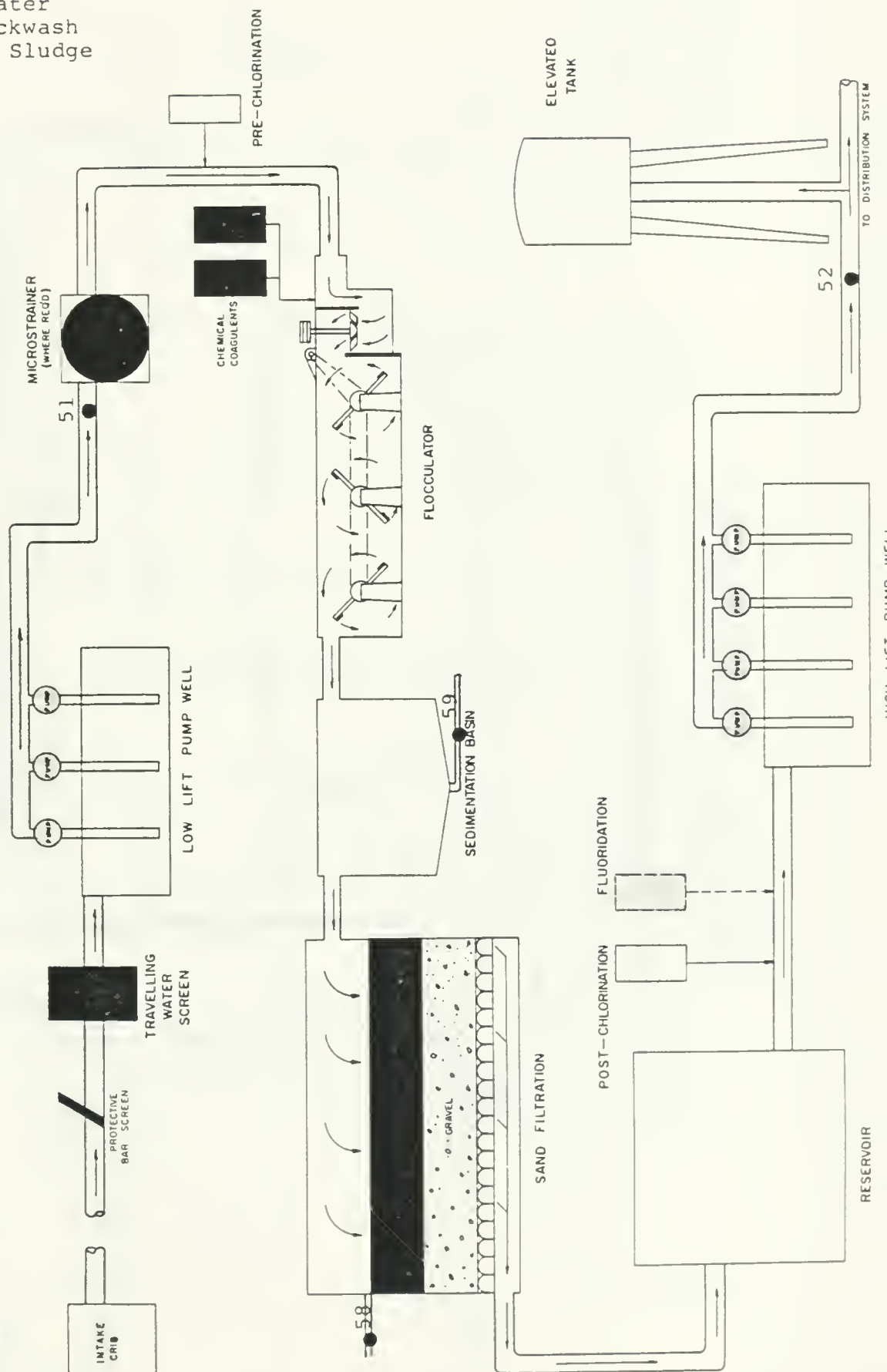


Figure 6-1 Sample Locations (Water)

# SEWAGE TREATMENT

## BY THE

### ACTIVATED SLUDGE PROCESS

- 03 Raw Sewage
- 04 Plant Effluent
- 05 Primary Effluent
- 05A Aeration M.L.
- 08 Return A.S.
- 15 Raw Sludge
- 16 Primary Dig.
- 17 Secondary Dig.
- 18 Dig.-Supernatant
- 23 Agr.
- application

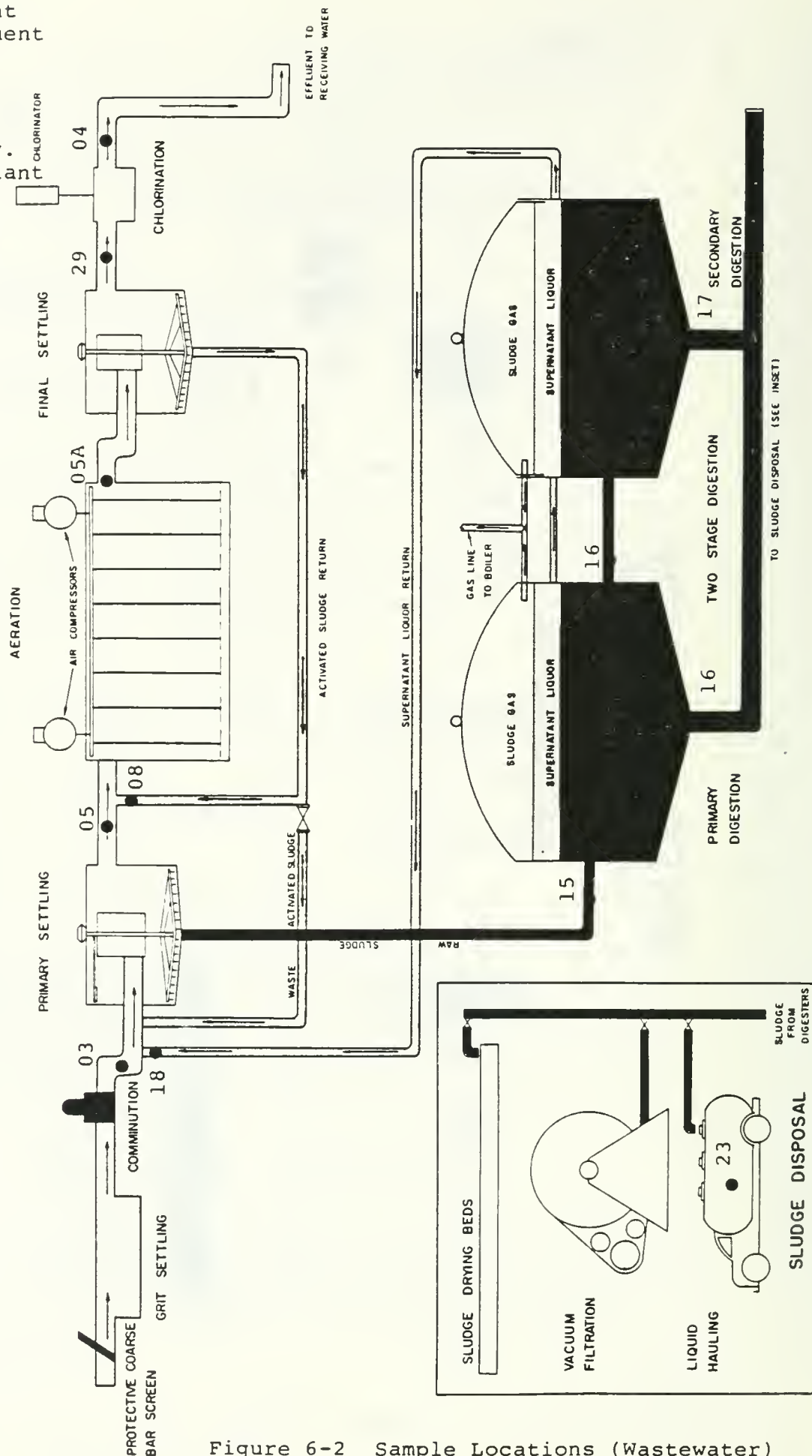


Figure 6-2 Sample Locations (Wastewater)

3) Filter Effluent

Grab samples are collected at this point on the same time schedule as the filter influent sample.

4) (52) Treated Water

This grab sample would normally be taken where the final chlorinated water enters the distribution system. If the water distribution system includes a reservoir or standpipe, an additional grab sample is normally taken downstream of these storage facilities.

B. Wastewater Treatment

1) (03) Raw Sewage, (05) Primary Effluent,  
(04) Plant Effluent

Composite samples should be taken wherever possible when sampling raw sewage, primary effluent and/or plant effluent. A continuous 24-hour sampling period is desirable, and the sample taken should be proportional to the flow. An automatic sampler should be used.

Because of the variability in raw sewage, it is important that frequent aliquots be obtained of the raw sewage and primary effluent. The same frequency is not as important when sampling plant effluent.

2) (15) Raw Sludge

The composition of raw sludge can vary widely between

pumping cycles, even within a single pumping cycle. To get a representative picture of the sludge, use a sample composed of at least three equal-sized grab samples. Take one grab sample at the beginning of one pumping cycle, another grab sample near the middle of a second pumping cycle, and a third grab sample near the end of a third pumping cycle.

3) Anaerobic Digested Sludge - (16) Primary Digestion

The primary anaerobic digester is usually heated and the contents well mixed and nearly homogeneous. A grab sample should be enough to determine the quality of the sludge, and the sample can be taken at some convenient location, if it is representative.

4) Anaerobic Digestion Sludge - (17) Secondary Digestion

A sample from the secondary digester can be taken when the sludge is removed from the tank for disposal or haulage. If possible, a composite sample should be taken, made up of one grab sample at the beginning of the day's pumping, another in the middle, and the third sample taken near the end of the pumping.

5) (18) Digester Supernatant

A sample is taken whenever the supernatant is withdrawn. A composite sample of at least three equal-sized aliquots should be taken: at the beginning, the middle and near the end of the recycle.

6)    (05) Aeration Tank (Mixed Liquor)

The aeration tank sample is taken at the effluent end of the aeration system. By necessity, it is a grab sample, taken at about the same time each day to ensure similar hydraulic conditions, (preferably mid-way through peak load conditions).

If there is more than one aeration tank, a sample should be taken from each tank.

For return sludge, a grab sample is enough to estimate the sludge solids concentration for wasting purposes.

7)    Aerobic Digester

The contents are usually well mixed - a grab sample should do it.

COLLECTION OF BACTI SAMPLES

Sampling of process flows for microbiological analysis requires special techniques not applicable to chemical or physical analyses. For "Bacti" sampling, a clean, rinsed bottle is not adequate for sample collection; pre-sterilized bottles must be used. In addition, the lab operator must use aseptic techniques when handling these sterile bottles. Inaccurate results are obtained when contamination occurs through faulty handling of sterile containers. The following paragraphs list a few of the basic principles of aseptic sampling technique:

## Surface Samples

Clamp the bottle onto a sampling pole before removing the cap. Touch only the outer surface of the cap when opening the bottle. The inner lip and liner must not come in contact with anything except the atmosphere. If the inner surfaces of the cap or bottle lip are accidentally touched, the sample has been contaminated and should not be submitted. It is a recommended procedure to hold the cap with your fingertips until the sample has been taken. The cap must not be set down somewhere while the sample is being taken as this will result in contamination.

Surface sampling is accomplished by quickly lowering the sample bottle below the surface of the water and holding it there until air bubbles have stopped coming to the surface. When sampling a stream or river place the bottle with the mouth facing into the current. When sampling near shore, care should be taken to get a sample uncontaminated with sediment. The bottle is then removed from the water, the level adjusted to the top of the label, immediately capped, and then unclamped from the sampling pole. Samples must be collected using this prescribed technique. The use of a dipper or other sampling device will result in contamination.

## Depth Samples

Depth samples are taken using sterile sampling bulbs. Bulbs should be used as quickly as possible: generally, within a matter of weeks, otherwise the rubber will crack and the bulb will not open. The same care that is used with sampling bottles must be used in the handling of bulbs. The glass plugs supplied have been sterilized within a cellophane envelope and must not come in contact with any contaminated surfaces when they are being removed

from the cellophane envelope. If, for some reason, the sampler should run out of glass rods, he may dip the metal plug into alcohol and flame it. After flaming, the plug is immediately inserted into the bulb, taking the usual precautions when handling sterile equipment. The use of the metal plug is discouraged and it should only be used in rare instances when the sampling could not possibly be obtained at a later time in the correct manner.

### Tap Samples

Samples from taps must be taken only after aerators, screens, hoses, etc. have been removed. Prior to sampling from a tap, the water should be allowed to run at a full flow for approximately two minutes. The strong flow will clean out residual contamination around the orifice of the tap thus providing for a more representative sample. The water pressure may then be reduced to permit taking the sample without excessive splashing which could result in contamination of the sample.

Fill the bottle to the top of the label being certain that the mouth of the bottle does not come in contact with the tap or any contaminated surface. The cap must also be handled aseptically as described previously.

### Perishability

While being transported to the laboratory, samples undergo changes (increases or decreases) in bacterial population, depending upon the initial bacterial density, the presence of growth-promoting or toxic substances in the water and the environmental conditions under which the samples are held. Analyses should be performed as soon as possible after collection, preferably within six hour, but not exceeding 24 hours.

Drinking water, waste and river samples that require transportation by public carriers received 24 to 72 hours after collection can be analyzed. Samples received after three days are rejected. Negative coliform results for drinking water samples arriving more than 48 hours after collection should be considered incorrect and fresh samples should be collected.

#### Sample Containers and Preservation Techniques

Pre-sterilized 180 ml bottles usually provide adequate volume (150 ml) for the routine analyses. Water from water treatment plants, distribution systems, sewage treatment plants and any water that has been chlorinated or in which a chlorine residual is suspected should be sampled in pre-sterilized 180 ml bottles containing sodium thiosulphate as a preservative. Sodium thiosulphate will neutralize the disinfecting capabilities of the chlorine thereby preserving the existing microbial population at the time of sampling. Samples collected for sulphur cycle bacteria analyses should be collected in sample bottles without sodium thiosulphate unless otherwise instructed by Microbiology personnel.

All samples should be collected early in the week and shipped to the appropriate laboratory without delay. During the Spring, Summer and Fall, samples must be packed in ice in order to minimize biological activity. During the winter, samples should be placed in an insulated container that will prevent freezing while still keeping them cold.

#### SAMPLE PRESERVATION

Complete and unequivocal preservation of samples, whether domestic sewage, industrial wastes, treated or

natural waters, is a practical impossibility. Regardless of the nature of the sample, complete stability for every constituent can never be achieved. At best, preservation techniques can only retard the chemical and biological changes that inevitably continue after the sample is removed from the parent source. The changes that take place in a sample are either chemical or biological. In the former case, certain changes occur in the chemical structure of the constituents that are a function of physical conditions. Metal cations may precipitate as hydroxides or form complexes with other constituents; cations or anions may change valence states under certain reducing or oxidizing conditions; other constituents may dissolve or volatilize with the passage of time. Metal cations may also adsorb onto surfaces (glass, plastic, quartz, etc.), such as, iron and lead. Biological changes taking place in a sample may change the valence of an element or a radical to a different valence. Soluble constituents may be converted to organically bound materials in cell structures, or cell lysis may result in release of cellular material into solution. The well known nitrogen and phosphorus cycles are examples of biological influence on sample composition.

Methods of preservation are relatively limited and are intended generally to (1) retard biological action, (2) retard hydrolysis of chemical compounds and complexes and (3) reduce volatility of constituents.

Preservation methods are generally limited to pH control, chemical addition, refrigeration, and freezing. Table 6-1 shows that various preservatives that may be used to retard changes in samples.

<u>PARAMETER</u>	<u>CONTAINER</u>	<u>PRESERVATION TECHNIQUE</u>	<u>MIN. VOLUME REQUIRED</u>	<u>COMMENT OR COMMON NAME</u>
<u>MAJOR IONS</u>				
Alkalinity	Glass or Plastic		75 ml	
Calcium	" "		75 ml	
Chloride	" "		50 ml	
Conductivity	" "		75 ml	Specific Conductance
Hardness	" "	None	75 ml	
Magnesium	" "		75 ml	
Potassium	" "		40 ml	
Silicates-Reactive	Plastic Only		50 ml	Silica
Sodium	Glass or Plastic		40 ml	
Sulphate	" "		50 ml	
<u>NUTRIENTS</u>				
Ammonia Nitrogen (Filtered)				Free Ammonia
Nitrate Nitrogen (Filtered)				
Nitrite Nitrogen (Filtered)	Glass or Plastic (polystyrene not linear polyethy- lene)	Freeze or Refrigerate	75 ml	
Phosphorus-Total				
Phosphorus-Filt- ered Reactive				Soluble Phosphorus, Orthophosphate
Phosphorus-Filt- ered Total	Screw-cap culture	Filter in field	35 ml	

TABLE 6-1

Sample Preservation Techniques

<u>PARAMETER</u>	<u>CONTAINER</u>	<u>PRESERVATION TECHNIQUE</u>	<u>MIN. VOLUME REQUIRED</u>	<u>COMMENT OR COMMON NAME</u>
<u>METALS</u>				
Aluminum				
Barium				
Beryllium				
Cadmium				
Chromium				
Cobalt				
Copper				
Iron				
Lead				
Lithium				
Magnesium	Plastic or Glass	HNO <sub>3</sub> to pH	100 ml	
Molybdenum		2 (approx.		
Nickel		20 drops)		
Silver		(1)		
Strontium				
Thallium				
Titanium				
Uranium				
Vanadium				
Zinc				
Antimony-Total	Plastic or Glass	None	100 ml	
Arsenic	" "	None	50 ml	
Boron	Plastic only	None	100 ml	
Chromium-	Glass only	None	100 ml	
hexavalent				
Mercury	Glass only	HNO <sub>3</sub> + KMnO <sub>4</sub> <sup>3</sup>	176 <sup>3</sup> ml	
Selenium	Plastic or Glass <sup>2</sup>	None	100 ml	

Note

- <sup>1</sup> Nitric acid preservative should be added AFTER the sample is placed in the bottle. If the sample contains visible suspended solids or where a hazardous chemical reaction between the sample and the acid may occur, submit the sample unpreserved.

TABLE 6-2

Sample Preservation Techniques  
(Con't)

<u>PARAMETER</u>	<u>CONTAINER</u>	<u>PRESERVATION TECHNIQUE</u>	<u>MIN. VOLUME REQUIRED</u>	<u>COMMENT OR COMMON NAME</u>
<u>ORGANIC</u>				
Alcohols	Glass	Refrigerate	1 litre	
Anionic Detergents - see MBAS, below				
Aromatic Hydro- carbons	Glass	"	1 litre	
Benzene Soluble Organics				
Biochemical Oxygen Demand	Glass	"	500 ml	BOD <sub>5</sub>
Carbon-Free (Elemental)				
Carbon Dioxide	Special	"	-	Free CO <sub>2</sub>
Carbon-Dissolved Inorganic	Glass or Plastic	"	50 ml	
Carbon-Dissolved Organic	"	"	50 ml	
Carbon-Inorganic	"	"	50 ml	
Carbon-Total	"	"	50 ml	
Carbon-C <sup>14</sup>				
Chemical Oxygen Demand	Glass	"	25 ml	C.O.D.
Fatty Acids - Volatile - Citric Acid - Maleic Acid - Phthalic Acid	Glass	Adjust to pH 3 with HCl; refrigerate	1 litre	
Foams	Glass	Refrigerate		
Freons	Glass			
Hexachloro- benzene	Glass	"		
Hydrocarbon Gases	Glass	"	1 litre	

TABLE 6-3

Sample Preservation Techniques

<u>PARAMETER</u>	<u>CONTAINER</u>	<u>PRESERVATION TECHNIQUE</u>	<u>MIN. VOLUME REQUIRED</u>	<u>COMMENT OR COMMON NAME</u>
<u>ORGANIC (Con't)</u>				
Mercaptans-see Volatile Sulfurous Organics				
Methane - see Hydrocarbon gases				
Methylene Blue Active Substances	Glass	"	100 ml	MBAS,ABS, LAS, Detergents
Petroleum Hydro- carbons(Gasoline)	Glass	"	1 litre	
Phenolics-Reactive	Glass	$\text{CuSO}_4 - \text{H}_3\text{PO}_4$	176 ml	
Polybrominated Biphenyls	Glass	Refrigerate	(2)	PBB
Polychlorinated Biphenyls	Glass	"	(2)	PCB
Polynuclear Aromatic Hydrocarbons	Glass	"	1 litre	PAH
Resins and Fatty Acids	Glass	Adjust to pH 3 with HCl; refrigerate	1 litre	
Tannins and Lignins	Glass	Refrigerate	200 ml	Either Solubles
Tracer Dyes				
Vinyl Chloride	Glass	"	1 litre	
Volatile Acids	Glass	"	25 ml	(Combined, for sewage sludges)
Volatile Sulfurous Organics	Glass	"	1 litre	

TABLE 6-4

SAMPLE PRESERVATION TECHNIQUE  
(con't)

<u>PARAMETER</u>	<u>CONTAINER</u>	<u>PRESERVATION TECHNIQUE</u>	<u>MIN. VOLUME REQUIRED</u>	<u>COMMENT OR COMMON NAME</u>
<u>OTHER</u>				
Acidity	Glass or Plastic	Refrigerate	50 ml	
Asbestos	Plastic	" Elapsed time between sampling & analysis must be less than 48 hours	1 litre	
Chlorine				
- Total Residual	Glass	(1)	500 ml	
- Free Available	"	(1)	500 ml	
- Monochloramine	"	(1)	500 ml	
- Dichloramine	"	(1)	500 ml	
Chlorophyll	Field filtration preferred	5 drops 10% MgCO <sub>3</sub> per liter prior to filtration	500 ml	
Colour-Apparent	Glass or Plastic	Refrigerate	75 ml	Hazen Colour Units
" -Dilution	" "	"	50 ml	
Cyanide	" "	NaOH to pH 11	500 ml	
Dustfall	See text, pg. 13			
Fluoridation Rate	See text, pg. 15			
Fluoride	Glass or Plastic	Refrigerate	50 ml	
Loss on Ignition	" "	"	500 ml	
Oxygen-Dissolved	Glass	(1)	1 liter	
Particles Size Analysis	Sediment samples only			
Particles Size by Microscopy	Non-aqueous samples only			
pH	Glass or Plastic	Refrigerate	50 ml	
Settleability	Glass	"	900 ml	
Sieve Analysis	Non-aqueous samples only			
Silicon	" "			

TABLE 6-5

Sample Preservation Techniques  
(con't)

<u>PARAMETER</u>	<u>CONTAINER</u>	<u>PRESERVATION TECHNIQUE</u>	<u>MIN. VOLUME REQUIRED</u>	<u>COMMENT OR COMMON NAME</u>
<u>OTHER</u> (Con't)				
Sludge Volume Index	-	-	-	Calculated parameter
Solids-Filtered	Glass	Refrigerate	75 ml	
Solids-Ignited	"	"	1 liter	
Solids-Suspended	"	"	600 ml	
Solids-Total	"	"	75 ml	
Sulfation Rate	See text, pg. 15	"		
Sulfide	Glass or Plastic	Zn acetate + Na <sub>2</sub> CO <sub>3</sub>	900 ml	
Sulfite	Glass or Plastic	Refrigerate	1 liter	
Sulfur-Total	Non-aqueous samples only			
Sulfur-Trace by S <sup>35</sup> dilution	Consult with Physical Methods Section			
Suspended Air Particles-Total	H1-Vol filters only			TSP
Thiocyanate	Glass or Plastic	NaOH to pH 11	1 liter	
Turbidity	" "	Keep in darkness	50 ml	

(1) Due to the perishable nature of the measured constituents, analysis should ideally be performed on-site. For lab analysis, after proper sampling and refrigeration, samples must be submitted within 4 hours of collection with prior laboratory notification.

TABLE 6-6  
Sample Preservation Techniques  
 (Con't)

<u>PARAMETER</u>	<u>CONTAINER</u>	<u>PRESERVATION TECHNIQUE</u>	<u>MIN. VOLUME REQUIRED</u>	<u>COMMENT OR COMMON NAME</u>
<u>MICROBIOLOGY</u>				
<u>Fecal Pollution Indicators</u>				
<u>Routine</u>				
Coliforms-Total	Presterilized Glass Bottles	Retrigration; Thiosulphate as re- quired for chlorinat- ed samples (see text)	150	Membrane Filtrat- ion.Incubation on Selective Agar
-Fecal	"	"	"	"
Fecal Streptococci	"	"	"	"
Presence/Absence Procedure	"	"	"	"
Pseudomonas aeruginosa	"	"	"	"
<u>Non-routine</u>				
Escherichia coli	"	"	"	"
Salmonella	"	"	500 - 1000	Membrane Filtration or Moore Swabs - Incubation in Selective Broth
<u>Organic Enrich- ment Indicators</u>				
Fungi	"	"	150	Membrane Filtration
Heterotrophic Bacteria				
- Surface Water	"	"	"	Spot or Spread Plate
- Treated Water	"	"	"	Incubation on Non- Selective Agar
Nuisance Organisms	"	"	"	Direct Microscopy or MPN Incubation in Selective Broth
Taxonomy	"	"	"	Direct Microscopy & Biochemical Testing

TABLE 6-7  
Sample Preservation Techniques  
 (con't)

<u>PARAMETER</u>	<u>CONTAINER</u>	<u>PRESERVATION TECHNIQUE</u>	<u>MIN. VOLUME REQUIRED</u>	<u>COMMENT OR COMMON NAME</u>
<u>MICROBIOLOGY (Con't)</u>				
<u>Specific (Industrial/ Agricultural) Pollution Indicators</u>				
Klebsiella	Presterilized Glass Bottles	Refrigeration; Thio- sulphate as required for chlorinated samples (see text)	150	Membrane Filtration Incubation on Selective Agar
Phenol degraders	"	"	"	MPN Incubation in Selective Broth
Nitrogen Cycle Bacteria				
- Nitrosomonas	"	"	"	"
- Nitrobacter	"	"	"	"
- Denitrifying Bacteria	"	"	"	"
Sulphate Reducers	"	Refrigerate only	"	"
Sulphur Oxidizers				
- Thiobacillus Ferrooxidans	"	"	"	"
- Thiobacillus Thioparus	"	"	"	"
- Thiobacillus Thiooxidans	"	"	"	"

TABLE 6-8

Sample Preservation Techniques  
(Con't)



SUBJECT:

TOPIC: 7

MICROBIOLOGICAL TECHNIQUES

**OBJECTIVES:**

The student will be able to:

1. Describe what is meant by P/A, MF and M.P.N. tests on potable water.
2. Describe briefly how each of the above tests is performed.
3. List the five main groups of Protozoa commonly used as indicator organisms in the Activated Sludge process, in their correct order of appearance from start up.
4. Describe the significance of filamentous bacteria in the Activated Sludge Process.
5. Recognize and indicate a healthy Activated Sludge sample when compared to a less desirable one, given a description of the relative numbers of indicator organisms present.



## MICROBIOLOGICAL INDICATORS

Examination of water samples for microbiological life performs an important process control function for both water treatment and wastewater treatment processes.

In water treatment plants, we use microbiological analysis as a quality control test. The degree of efficiency of the disinfection stage of our process is measured indirectly by our success at inactivating an "indicator organism" group of bacteria. By monitoring the number of these organisms in our treated effluent, we are able to predict the degree of success we are having at inactivating other, more harmful species.

In wastewater treatment plants, we are able to use certain Protozoa species to indirectly indicate the bacterial growth conditions present in our Activated Sludge Process. The relative numbers of a group of Protozoa "indicator organisms" present in a sample of aeration tank mixed liquor can tell us whether the ratio of food to micro-organisms (F/M) has been adjusted to give the best growth conditions possible. The indicator Protozoa therefore can give us information about the state of health of the Activated Sludge Process (a biological oxidation process).

Let us look at the microbiological analyses performed on water samples first.

### A. MICROBIOLOGICAL ANALYSIS OF POTABLE WATER

#### Scope

A number of parameters are available for the identification and evaluation of different problems. When water

is being tested for its suitability from a health standpoint (for the possible presence of pathogenic bacteria), a standard membrane filtration method for total coliforms, fecal coliforms and fecal streptococci can be performed. It is also possible to determine the presence of pathogenic bacteria such as Pseudomonas aeruginosa and Salmonella sp. Total viable bacterial concentrations can be determined by the heterotrophic bacterial count (either spot or spread plate procedures).

A test developed for the analysis of drinking water is the presence-absence test, which will detect the presence of coliforms, fecal coliforms, fecal streptococci, aeromonads, pseudomonads and clostridia.

Samples collected for investigation of taste, odour and colour problems are analyzed for iron bacteria, sulphur bacteria and other pertinent nuisance bacteria.

In cases where surveys call for the evaluation of specific problems, such as industrial waste inputs, additional microbial parameters can be used. These include sulphur oxidizers, sulphate reducers, nitrifiers and denitrifiers.

## FECAL POLLUTION INDICATORS

### ROUTINE

#### Total Coliforms (TC) Procedure

The TC group is comprised of all of the aerobic and facultative anaerobic, Gram-negative, non-sporeforming, rod-shaped bacteria which ferment lactose with gas formation within 48 hours at 35°C or all organisms which produce a colony with a golden- green metallic sheen within 24 hours of incubation using the MF technique. All coliforms are oxidase negative. This definition includes, in addition to the intestinal forms of the Escherichia coli group, closely related bacteria of the genera Klebsiella, Citrobacter and Enterobacter. Enterobacter - Citrobacter groups are common in soil but are also recovered from feces in small numbers. Therefore, the presence of total coliforms in water is not a specific indicator of sewage or fecal pollution but may also indicate soil runoff.

#### Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml (6 ounces)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

TC levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours (in some cases 3 days is allowed but this procedure is not advised).

## Method

Analysis for TC is by membrane filtration according to the MOE Handbook of Analytical Methods for Environmental Samples (HAMES). A given volume of the sample or dilution is filtered using a  $0.45\mu$  gridded, white membrane filter (Gelman GN-6). The membrane is placed on m-Endo Agar LES (Difco) and incubated for  $22 \pm 2$  hours at  $35 \pm 0.5^{\circ}\text{C}$ . After incubation, the number of total coliforms and background colonies are counted using a stereoscopic microscope at a 10X magnification.

## Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required, the number of parameters requested per sample and the incubation periods for each parameter. TC results are generally available within 48 hours of submission.

## Fecal Coliforms (FC) Procedure

This FC group of bacteria is basically a subgroup of the Total coliforms and is comprised of aerobic and facultative anaerobic, Gram-negative, non-sporeforming, rod-shaped bacteria which ferment lactose at  $44.5^{\circ}\text{C}$ . The FC parameter is a more specific test for bacteria associated with human and animal fecal material. This test has proved useful as an indicator of relatively recent fecal pollution inputs, however, it is by no means completely selective for Escherichia coli, the coliform most directly related to fecal pollution. Bacteria related to other forms of input such as Klebsiella may be detected with this test.

## Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml - analysis performed on sample obtained for TC	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

FC levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours (in some cases 3 days is allowed but this procedure is not advised).

## Method

Analyses for FC is by membrane filtration (HAMES). The membrane is placed on a filter mTEC Agar (replacing MacConkey Membrane Broth) and incubated to allow a gradual increase to  $44.5 \pm 0.5^{\circ}\text{C}$  over approximately 4 hours. Total incubation time is  $22 \pm 1$  hours. After incubation, the number of FC colonies are determined with a stereoscopic microscope at 10X magnification. All yellow, yellow-brown and yellow-green colonies are counted.

## Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required, the number of parameters requested per sample and the incubation periods for each parameter. FC results are generally available within 48 hours of submission.

### Fecal Streptococci (FS) Procedure

The FS group of bacteria is comprised of aerobic, spherical to ovoid Gram positive cells occurring singly, in pairs or in short or long chains. When used as indicators of fecal contamination, the following species and varieties are implied: Streptococcus faecalis, S. faecalis var. liquefaciens, S. faecalis var. zymogenes, S. faecium, S. faecium var. durans, S. bovis and S. equinus. FS can best be used in conjunction with the FC parameter to indicate the nature of the fecal source. If the ratio of the geometric mean densities of FC to FS at pollution sources or outfalls exceeds 4, then the source is likely human in origin. A ratio of 0.7 or less suggests a non-human source. Ratios between these values are difficult to interpret and may be mixtures. Numerous environmental factors may influence the densities of FC and FS and thus care must be used in interpretation. This ratio is only valid when the FC density approaches or exceeds 100/100 ml.

### Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml - analysis performed on sample obtained for TC and FC	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

FS levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours (in some cases 3 days is allowed but this procedure is not advisable).

## Method

Analysis for FS is by membrane filtration (HAMES). The membrane is placed on m-Enterococcus Agar (Difco) and incubated at  $35.0 \pm 0.5^{\circ}\text{C}$  for  $48 \pm 3$  hours. Using a stereoscopic microscope (10X), all colonies that are red, maroon or pink are counted as fecal streptococci.

## Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required, the number of parameters requested and the incubation periods for each parameter. FS results are usually available within 72 hours of submission.

## Presence-Absence (P-A) Procedure

The P-A procedure has been developed as an alternative to the membrane filtration procedure and is a sensitive means of detecting pollution indicator bacteria in drinking water samples. Essentially, the P-A test is a modification of the most probable number (MPN) procedure, which uses a dilution series of broth tubes to detect and enumerate microorganisms. The P-A test employs only one analysis bottle per sample. The test allows for an inoculum of up to 100 ml and will qualitatively determine a wide variety of pollution indicator bacteria. They include total coliforms, fecal coliforms, fecal streptococci, Pseudomonas aeruginosa, Staphylococcus aureus, Aeromonas sp. and Clostridium perfringens.

### Sampling

Minimum Volume of Sample	Required Sample container	Special Handling Technique
150 ml (6 ounces)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

Bacterial levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within 6 hours, but not exceeding 24 hours if feasible. Sample analysis will not be performed on samples older than 3 days.

### Method

The P-A test involves the inoculation of 50 or 100 ml of water into a bottle of MacConkey broth (HAMES). The bottle is incubated at  $35 \pm 0.5^{\circ}\text{C}$  for up to four days. If growth occurs with the production of acid and/or gas, then other tests are used to determine the types of bacteria present.

### Time of Analysis

The time required for completion of test procedures associated with the P-A test is up to one week depending on the level of pollution in the sample. A sample containing a variety of indicator bacteria will require several confirmation tests before results can be reported. Drinking water samples which show the presence of fecal bacteria within 24 hours will have this information phoned to the municipality as soon as confirmed results are available.

### Pseudomonas aeruginosa (P. aeruginosa) Procedure

P. aeruginosa is a motile, Gram-negative rod which does not ferment lactose and is oxidase positive.

Isolates of P. aeruginosa are found in human feces and their presence in water is generally the direct cause of recent fecal waste inputs from a nearby source. Their presence is thus a good indication of a potential health hazard.

P. aeruginosa is also an opportunistic pathogen and has been identified as the causative agent of a number of infections that may be transmitted through a contaminated water body to a susceptible host. Of chief concern to bathers is the association between P. aeruginosa and the disease known as otitis externa "swimmer's ear".

#### Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml (6 ounces)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

P. aeruginosa levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours.

### Method

An appropriate volume or dilution of the sample is filtered through a 0.45  $\mu$  membrane filter and incubated on mPA Agar at  $41.5 \pm 0.5^{\circ}\text{C}$  for  $48 \pm 2$  hours..

Using a stereoscopic microscope (10X), all tan to dark-brown or black colonies are counted as Pseudomonas aeruginosa.

### Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required, the number of parameters requested and the incubation periods for each parameter. Consultation is necessary prior to this parameter being requested. P aeruginosa results are usually available 72 hours after submission.

## NON-ROUTINE

### Escherichia coli (E. coli) - Experimental Procedure

E. coli is the predominant, facultative bacterial species in the large bowel and is thus the coliform most directly related to fecal pollution. E. coli is occasionally pathogenic to man (e.g. urinary tract infections) but its primary importance in water bacteriology is as an indicator.

Methods developed historically for the detection of fecal pollution have not been sufficiently specific for E. coli. However, a recently developed medium designed specifically for the detection of E. coli has been tested in this laboratory and proven successful. It is now possible to determine concentrations of E. coli on a limited number of samples.

This technique can only be performed with prior consultation and is not available for routine work.

#### Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml (6 ounces)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water.

E. coli levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours.

## Method

Analysis for E. coli requires filtration of an appropriate volume or dilution of the sample and incubation on mTEC Agar. The plates are incubated at  $35 \pm 0.5^{\circ}\text{C}$  for two hours followed by  $22 \pm 1$  hours at  $44.5 \pm 0.5^{\circ}\text{C}$ . The filter is then transferred to a filter pad saturated with urease reagent and allowed to stand for 15 minutes. Urease positive colonies will turn magenta. Using a stereoscopic microscope (10X), all colonies that remain yellow, yellow-green or yellow-brown, are counted as E. coli.

## Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required, and the incubation period. E. coli results will be available 2 days after sample submission.

## Salmonella

Salmonella is one of the most important and common waterborne pathogenic organisms. The members of this genus are widely distributed in the environment and are probably responsible for most of the recognized waterborne disease outbreaks. Salmonella can be frequently detected in sewage, stormwater and polluted rivers and lakes. Fecal contamination from animals, particularly domestic, and insufficiently treated human wastes are the major source of these organisms.

Since salmonella serotypes are all potentially pathogenic, their frequent isolation is taken as proof of a public health hazard.

The methods required are complex and slow, therefore their use is recommended only for specific studies and only with careful consultation and planning.

## Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
500-1000 ml (18-36 ounces)	Pre-sterilized glass bottles	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

Sampling can also be carried out with Moore Swabs (gauze pad) placed at appropriate locations in sampling areas.

Salmonellae levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours.

## Method

There are several procedures for the isolation and identification of salmonellae from waters and wastewaters. The method chosen will depend on the survey conducted. All the procedures include sample concentration (Moore swab or membrane filtration), enrichment in selective broths, plating on selective agar media and identification by a variety of biochemical and serological tests.

## Time of Analysis

The number of samples analyzed will depend on the volume and type of sample and, more specifically, on the methods necessary for analysis. In general, a five-day period is required for Salmonella analyses.

Consultation with Microbiology staff prior to the use of this parameter is absolutely essential.

## ORGANIC ENRICHMENT INDICATORS

### Fungi (Yeasts and Molds)

Fungi are ubiquitously distributed, achlorophyllous, heterotrophic microorganisms which are present wherever living or nonliving organic matter occurs. Both saprophytic and pathogenic fungi have been found in sewage and sludge, domestic and industrial effluent and marine and freshwater environments. The densities and distribution of species of fungi in such habitats can be used as an index of the degree of organic enrichment. In addition, the presence of certain pathogenic fungi (e.g. Candida albicans) in recreational waters is useful in assessing water quality and in evaluating public health hazards.

### Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml (6 ounces)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

Fungal levels, yeasts in particular, will undergo change even upon refrigeration, therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours.

### Method

No standard techniques have been developed for quantitative enumeration of fungi from an aquatic environment. In general, selective agar media, the membrane filtration technique and the spread plate technique are used. Fungal densities are expressed as colony-forming units (CFU) per 100 ml.

### Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required and the incubation periods. In general, the analysis requires a five-day period. Prior consultation is required before this parameter can be requested.

### Heterotrophic Bacteria (HB) - Surface water

The HB count is not a test for a specific microorganism. It is designed to enumerate as large a number as possible of those bacteria in water that require some organic carbon for their growth. The concentration of heterotrophic bacteria in water is affected by the levels of organic and inorganic nutrients present. Densities of HB in lakes of high trophic status are higher than HB levels in oligotrophic lakes.

### Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
Can generally be done from sample for analysis of TC, FC and FS or <u>P. aeruginosa</u> (150 ml)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

HB levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours.

### Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required, and the incubation period. HB results by this method are available 8 days after sample submission.

### Method

Analysis is by the spot plate or spread plate method. Two volumes of 0.1 ml of the sample and/or sample dilution are placed on pre-dried CPS agar plates. The spread plate method requires one volume of 0.1 to 1.0 ml spread over the surface of the pre-dried agar plate. Plates are incubated at  $20 \pm 0.5^{\circ}\text{C}$  for 7 days. Using a stereoscope microscope (10X), a total colony count is obtained.

### Heterotrophic Bacteria (HB) - Treated Water

The HB parameter can also be used for the analysis of treated waters (i.e. drinking waters including treated surface waters, water in distribution systems, bottled water, untreated well water and bathing waters including treated beaches, pools, saunas and mineral springs). In these waters the HB parameter is used to measure treatment efficiencies and as an indicator of bacterial water quality. This procedure has not yet been introduced as a routine method and thus prior consultation is required before it is requested.

### Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
Can generally be done from sample analysis of TC, FC and FS or <i>P. aeruginosa</i> (150 ml)	Pre-sterilized glass bottles or rubber syringes	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water

HB levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 24 hours.

#### Method

Analysis is by the Spot Plate, Spread Plate, or membrane filtration if concentrations of HB are low. Standard Methods (14th Edition) recommends the use of tryptone glucose extract or plate count agar with an incubation period of  $48 \pm 3$  hours (bottled water for  $72 \pm 4$  hours) at  $35 \pm 0.5^{\circ}\text{C}$ . A total colony count is obtained using a stereoscopic microscope (10X).

#### Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of dilution steps required and the incubation period. HB results by this method would be available 3 to 4 days after the sample is submitted.

## NUISANCE ORGANISMS

Certain microorganisms, although not pathogenic or indicative of the presence of fecal material, may be related to a deterioration in water quality or may reduce the efficiency of the effective treatment of water or sewage. Foul tastes and odours, slimes, discolouration, bulking of activated sludge, and the clogging or corrosion of treatment or distribution systems may all result from the presence of one or more groups of microorganisms. Nuisance organisms include iron bacteria such as Gallionella, Leptothrix, Sphaerotilus and Crenothrix, the sulphate reducing bacteria of the genera Desulfovibrio and Desulfotomaculum, the sulphur oxidizer Beeggiatoa, actinomycetes, algae and fungi.

### Sampling

Minimum Volume of Sample	Required Sample Container	Special Handling Technique
150 ml (6 ounces)	Pre-sterilized glass bottles	Pack in ice immediately after sampling. Use bottles containing thiosulphate for chlorinated water*

\* Do not use thiosulphate bottles when presence of sulphate reducers is suspected. The sample should contain material representative of the problem such as slimes or filamentous growth collected aseptically.

Nuisance organism levels will undergo change even upon refrigeration; therefore, immediate delivery to the laboratory is essential. Analyses should be performed as soon as possible after collection, preferably within six hours, but not exceeding 48 hours.

### Method

With the exception of the sulphate reducing bacteria and fungi, identification is generally based upon direct microscopic observation of the sample. Morphological characteristics are generally used to identify the organism(s) present. The sulphate reducers may be cultivated and their presence revealed by the detection of metabolic byproducts. Fungi may also be cultivated and later identified by microscopic observation of their morphological characteristics.

### Time of Analysis

The number of analyses performed per day will depend on the type of sample, the number of parameters requested and the incubation periods (if necessary) for each parameter. Results of microscopic analyses are available two days after sample submission, however, because the method and number of analyses vary with the sample problem, some analyses may take one month to complete. Consultation with Microbiology staff is necessary prior to sample collection.

## B. MICROSCOPIC EXAMINATION OF ACTIVATED SLUDGE

### FUNCTIONS OF MICROORGANISMS

#### General

Activated sludge contains millions of microscopic forms of life. The specific contributions made by each of these types of living organisms, however, in the purification of wastewater are not firmly established. Of interest from a process control point of view are bacteria and protozoa. There are other organisms, such as rotifers, nematodes (roundworms) and waterborne insect larvae but these do not have any significance in the treatment process. See Figure 7-2. All of these living organisms require carbon in some form as energy, a small amount of which must be carbon dioxide; most also require some organic carbon compounds (such as sugars) and nitrogenous compounds as well as sulphur and phosphorus.

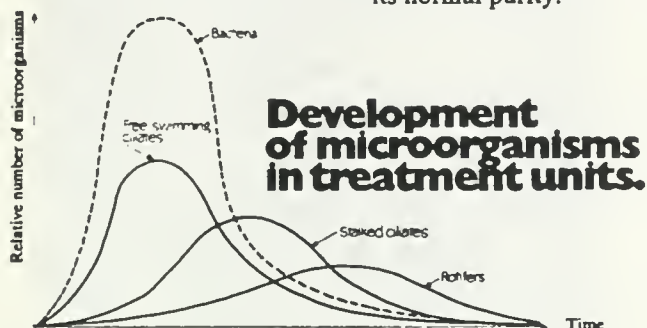
Stated in simple terms, the activated sludge process involves bringing together wastewater and a mixed culture of micro-organisms under aerobic conditions. Depending on the source of carbon used to create their cell mass, these micro-organisms are classed as autotrophic or heterotrophic.

1. Autotrophic - derive their cell carbon from inorganic carbon sources
2. Heterotrophic - derive their carbon from organic carbon sources.

Bacteria use the organic matter in wastewater for food and reproduction. The increased bacterial growth results in the accelerated extraction of wastes from solution, improved flocculation characteristics in the activated sludge and a biological floc with improved settling characteristics.

# How microorganisms function in biological waste systems

Wastes contain many varieties of living organisms. Discussed here are some of the beneficial types which break down and transform pollutants into harmless substances, thereby helping to return water to its normal purity.



## Biological floc

I = Active solids  
I + II = Volatile solids  
III = Inert solids  
I + II + III = Total solids

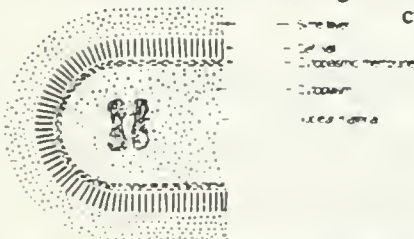


## Bacteria

Bacteria are microscopic one-celled plants. They are responsible for the purification of the organic pollution contained in waste and form the active part of floc. The bacterial species present, such as pseudomonas, flavobacterium, etc., are dependent on the nature of the organic matter to be decomposed. They develop rapidly in all biological treatment systems.

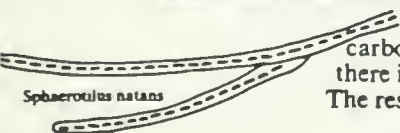
## Bacteria cell

In a bacterial cell, the organic matter must cross the cytoplasmic membrane before being utilized.



## Filamentous bacteria

These bacteria develop where carbohydrates are present and where there is low dissolved oxygen content. The result is bulking and poor settling.



## Fungi

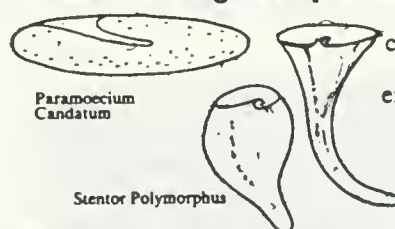
Like bacteria, these multicellular microscopic plants decompose soluble organic matter. Development is most rapid when the pH is acid and the oxygen level is low. Their presence is undesirable because they cause bulking.



## Protozoa

Unicellular microscopic animals, protozoa consume bacteria, thus promoting the growth of new bacteria. They feed on the surface of biological floc and on dispersed bacteria, which results in a clear effluent. The presence of protozoa indicates that there is sufficient dissolved oxygen and a lack of toxic elements. There are two basic forms:

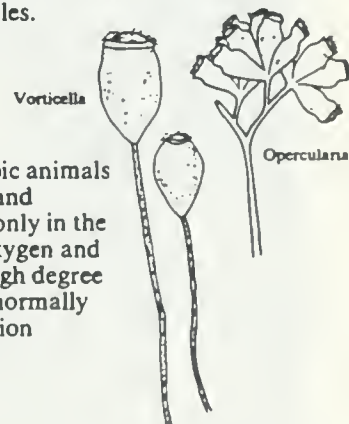
### Free-swimming ciliate protozoa



Fine hairs allow these ciliates to swim rapidly. They have a high energy level and require a large quantity of organic food.

### Stalked ciliate protozoa

Normally found in high-rate systems in equal numbers with the free-swimming ciliates, they attach themselves by their stalks to solid particles.



## Rotifers

Multicellular microscopic animals which feed on bacteria and protozoa, rotifers exist only in the presence of dissolved oxygen and are an indication of a high degree of treatment. They are normally found in extended aeration systems.



Philodina Roseola

Figure 7-1

HEAD OFFICE: 2015 Drummond Street, Montreal 107, Quebec  
BRANCH: Suite 215, Scotia Square, Halifax, N.S.  
AGENTS IN ALL PRINCIPAL CITIES.

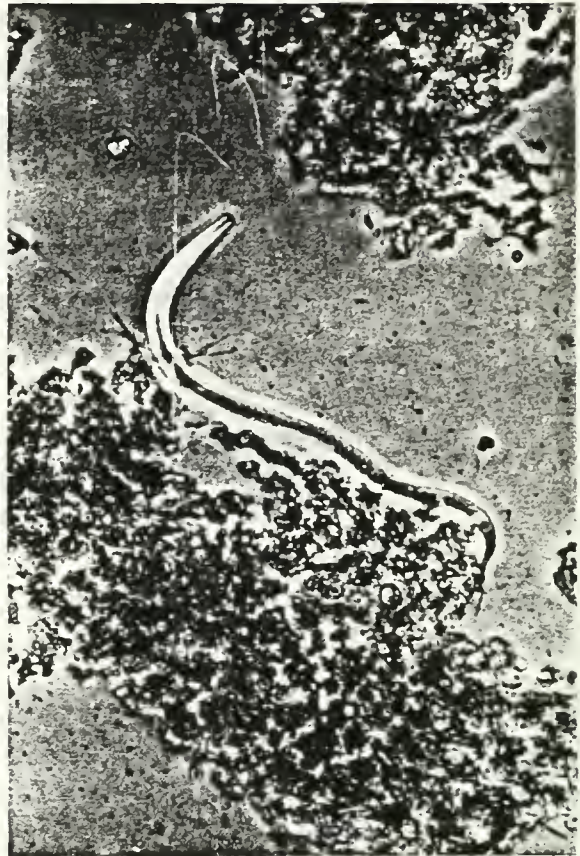


**Degremont**

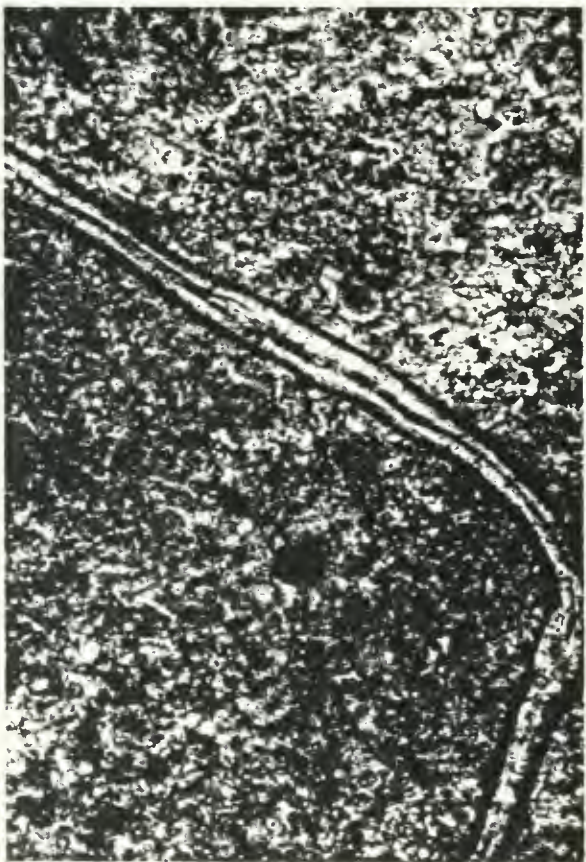
Figure 7-2  
MICROSCOPIC VIEW OF  
ROTIFERS, NEMATODES AND FIBRE



ROTIFER



NEMATODE (ROUNDWORM)



FIBRE (WOOD-CLOTH)

## Indicating Organisms

Because there is a progressive development of micro-organisms in treatment units from the initiation of an activated sludge to a well stabilized condition, the order in which the protozoa appear can be predicted. Varying sludge conditions will affect the order of appearance. Protozoa are therefore classed as indicating organisms. Rotifers on the other hand appear only when the wastewater treatment system is in an advanced state of stabilization. Since they can exist under conditions which are not compatible with the function and survival of the other organisms, the role of rotifers as an indicator organism is suspect. The presence of filamentous bacteria can also indicate, or forewarn of, process control problems.

The important aspect from an operation standpoint is the types of protozoa present. *There is a definite succession of protozoa as the sludge is formed and the process becomes efficient.* (See Figure 7-3). Knowing the various protozoa and their significance can be a valuable guide to better activated sludge operations. Protozoa can be observed quickly using a microscope and such observations reflect the immediate condition of the sludge.

By identifying the indicator organisms through regular microscopic examination of the activated sludge, the operator can recognize or even predict problems occurring or likely to occur in the treatment process.

## IDENTIFICATION AND EVALUATION

### Filamentous Organisms

Filamentous organisms are commonly associated with poor settling or "bulking" sludges, producing sludge volume indexes (SVI) as high as 500. The presence of these organisms is caused by many complex conditions (e.g. organic overloading) and may adversely affect the effluent quality.

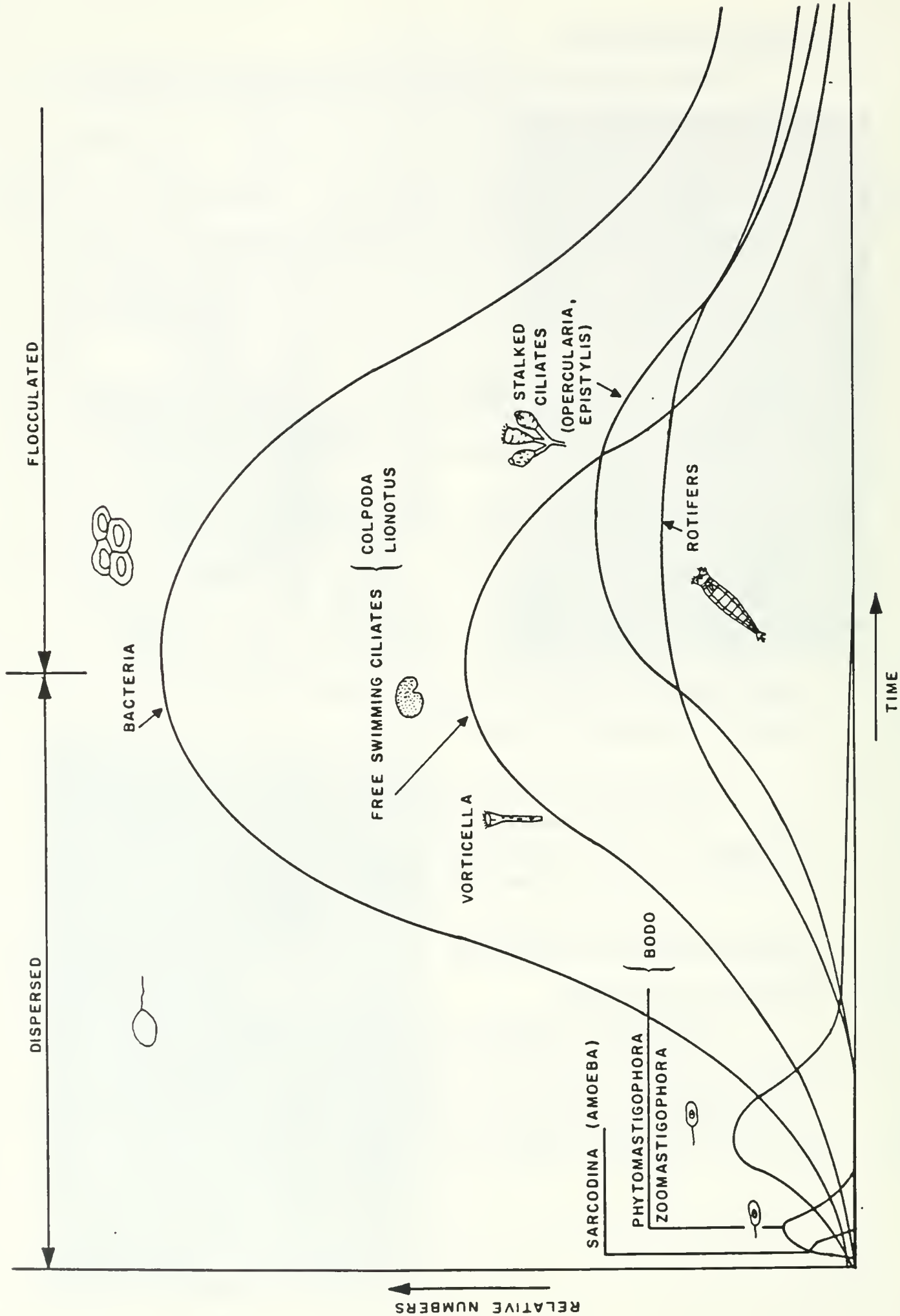


FIG. 7-3 - RELATIVE PREDOMINANCE OF MICROORGANISMS IN ACTIVATED SLUDGE SYSTEMS

In a mixed liquor, there are different types of filamentous organisms which may appear, when viewed at 100X, as a multitude of very fine strands haphazardly interwoven throughout the sludge. See Figure 7-4. Frequently, these filaments are of the *sphaerotilus* species. Also, when "sphaerotilus" is present, the effluent may be clear and sparkling because it does a good job of clarification. However, the sludge settles so badly that mixed liquor is lost over the weirs under normal hydraulic conditions.

### Protozoa

Protozoa are single-celled animals which are characterized by the fact that they are microscopic and that they ingest solid food, either alive or previously alive. The types which prevail in any aeration system vary, depending upon the nature of the wastes being treated. Curves in Figure 7-3 show successions of dominant protozoa in relation to the degree of purification. The species of protozoa present in the activated sludge changes with process conditions and effluent quality. Industrial waste systems or systems with a high influx of industrial wastes require careful control to ensure continuous operation at maximum efficiency. Protozoa are more easily affected than bacteria by toxic compounds and other adverse conditions such as oxygen deficiency or excessive organic loads, so regularly observing the changes in protozoa from their normal levels can give an immediate indication of an upset before it has had time to affect the bacteria.

*In industrial wastewater treatment plants, the kinds of protozoa may differ from those normally associated with municipal wastewater treatment plants.*

For the purpose of the role they play in sewage treatment we will consider three groups of subphylum of protozoa, *Sarcodina*, *Mastigophora* (*Flagellata*) and *Ciliophora* or *Ciliata*. These are considered below in the general order of their appearance in a new aeration system.

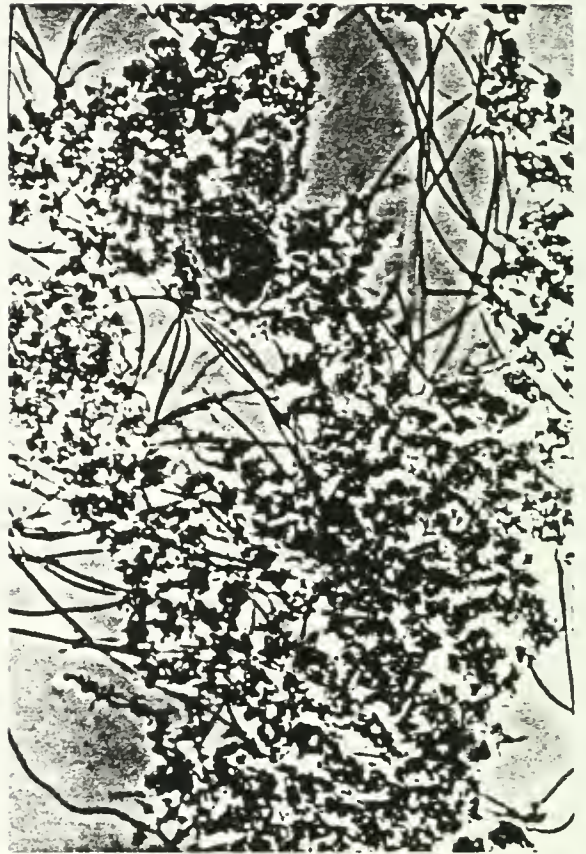


Figure 7-4  
MICROSCOPIC VIEWS OF FILAMENTOUS BACTERIA

1. Sarcodina (Figure 7-5)

The distinguishing feature of members of this group is the presence of pseudopodia (false feet). These tiny organisms have an extremely elastic cell membrane and the fluid protoplasm within the cell literally "flows" in the direction that the animal wishes to move. *Protoplasm* is the clear, jelly-like substance in all plant and animal cells. Pseudopodia will also be extended to surround food particles which are then ingested through the cell membrane. The amoeboids are common among this group.

*Sarcodina predominate in activated sludge systems during the start-up, or during the system's recovery from a kill.*

2. Mastigophora (Flagellata) (Figure 7-5)

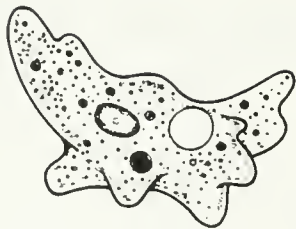
The members of this group are small round or elliptical organisms bearing flagella (tails). These are whip-like appendages which enable the organism to move through the water, usually with a corkscrew motion. At low magnifications (100X), the fast, erratic movement of the tail would not be visible. One or more species of the flagellata may be present; for example, bodo species, monas species.

*Mastigophora appear when the bacteria population is low and the food concentration high. As the bacteria increase in numbers, the flagellates cannot compete for the available food and they soon decrease and become insignificant.*

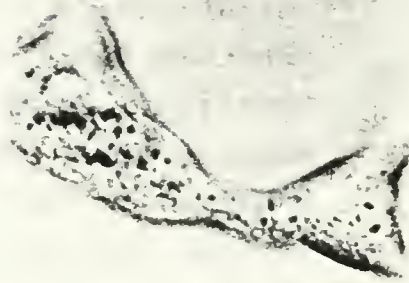
3. Ciliophora or Ciliata

These animals have very fine hairs called "cilia" which cover all or some of their bodies. Cilia are used for locomotion, and the cilia around the gullet are used for capturing food. Possession of cilia is common to all members of this group, although they are more easily seen in some species than in others.

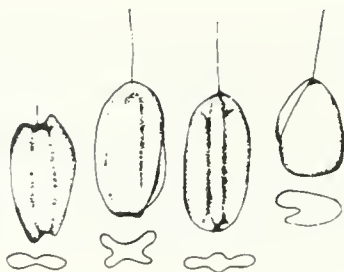
Ciliata are substantially larger than flagellates.



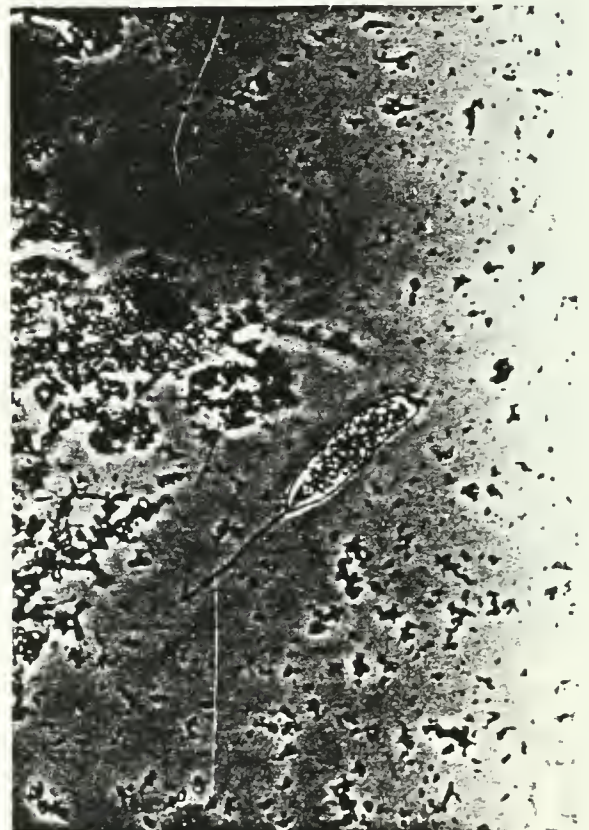
Sketch of Sarcodina  
(amoeba)



Microscopic View of Amoeba



Sketch of Mastigophora (Bodo)  
(Flagellates)



Microscopic View of  
Mastigophora (Euglena)

Figure 7-5  
SARCODINA AND MASTIGOPHORA

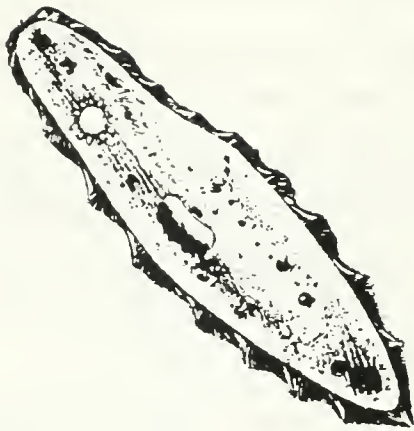
Ciliata may be broken down into three groups:

- a) The *free-swimming* ciliates, such as paramoecium colpeda and colpidium; (See Figure 7-6)
- b) The *crawling* types, of which aspidiscus will be the most common one; (See Figure 7-7)
- c) The *stalked* types, which are anchored to a clump of sludge and are either (i) individual; for example, vorticella; or (ii) colonial (grouped); for example, opercularia. (See Figures 7-7 to 7-9)

The presence of ciliophora is an indication of bacterial activity at any given time. The bacterial activity is a measure of the biochemical condition of the system. The ciliates reflect the efficiency of the process with considerable accuracy when observed with the microscope under various day-to-day loading conditions. For example, slug loads of chemical or organic wastes will be indicated by a noticeable reduction of ciliata present. The rise and decline of the types considered normal can indicate an upset some time before the bacteria can be knocked out, because the ciliates are more easily affected by toxicity and load changes.

*The free-swimming ciliata are present when there are numerous free-swimming bacteria. The ciliates feed on large numbers of these free-swimming bacteria, reducing the effluent turbidity and BOD to levels found in normal activated sludge systems. Large numbers of free-swimming and crawling ciliates indicate good operation. Flagellates and free-swimming ciliates may indicate low treatment efficiency, whereas the presence of stalked ciliates and some higher forms of life (for example, rotifers) should indicate an efficiently operating system.*

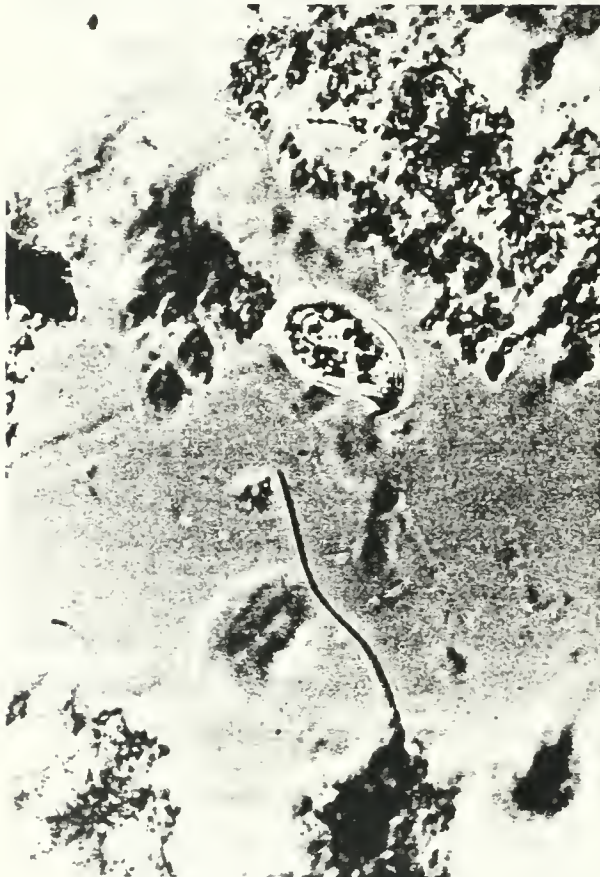
*Stalked ciliates arise when the free-swimming ciliates are unable to compete for the available food. An activated sludge system which is very stable will have stalked ciliates and usually very few spontaneously moving protozoans.*



Sketch of Paramecium  
(Free-Swimming)



Microscopic View of  
Colpidium (Free-Swimming)



Microscopic View of  
Colpoda (Free-Swimming)



Microscopic View of  
Lionotus

Figure 7-6 CILIATA (FREE-SWIMMING)



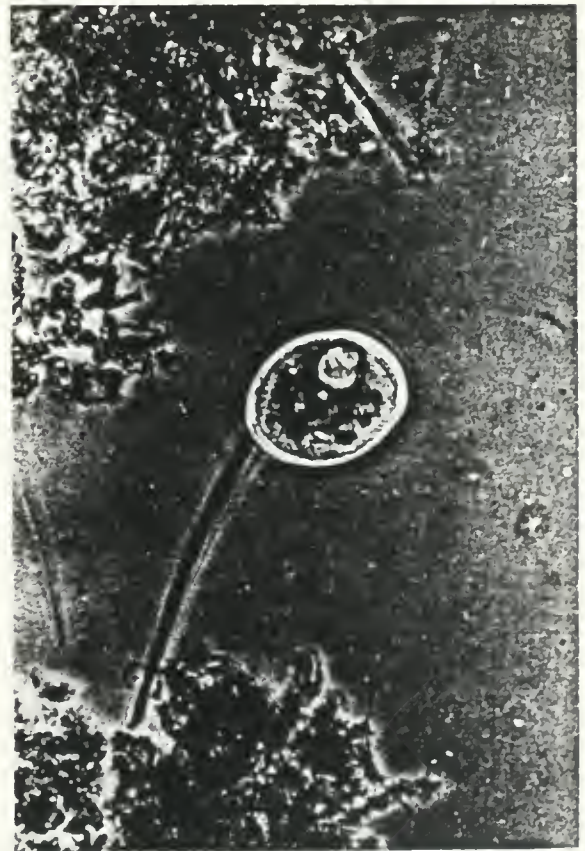
Microscopic View of  
Aspidiscus (Crawling)



Sketch of Vorticella  
(Stalked)



Microscopic View of  
Vorticella

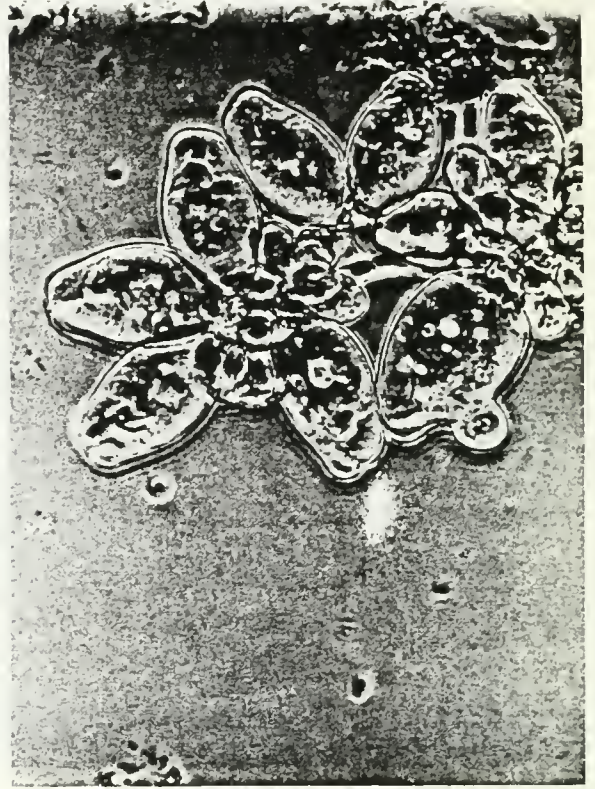


Microscopic View of  
Vorticella

Figure 7-7 CILIATA (STALKED AND CRAWLING TYPES)



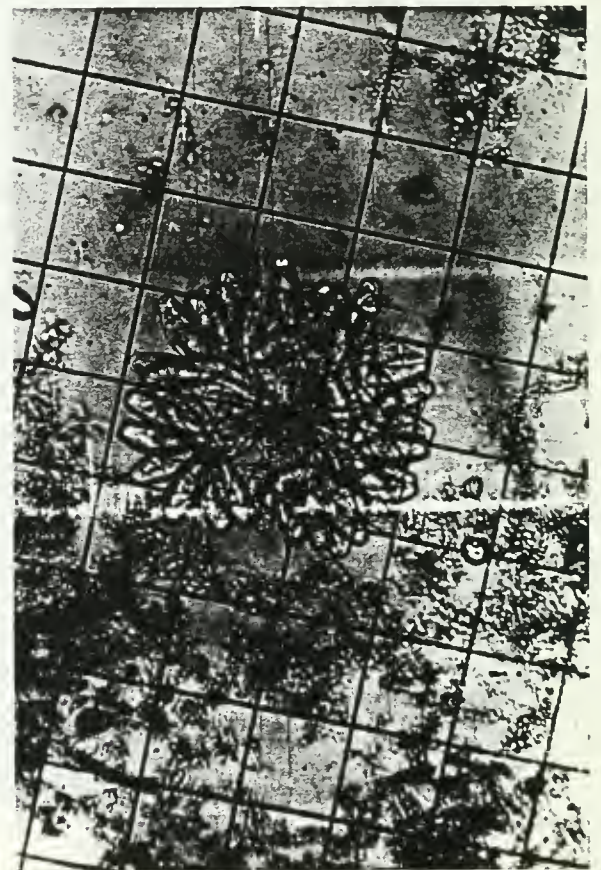
Sketch of Opercularia



Microscopic View of Opercularia



Sketch of Carchesium

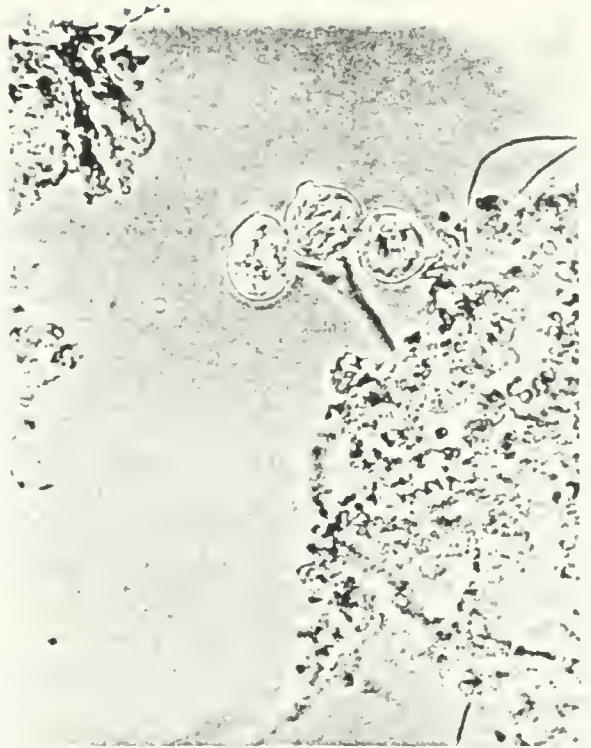


Microscopic View of  
Large Colony Opercularia

Figure 7-8 CILIATA STALKED TYPES



Sketch of Epistylis



Microscopic View of Epistylis

Figure 7-9 CILIATA STALKED TYPES

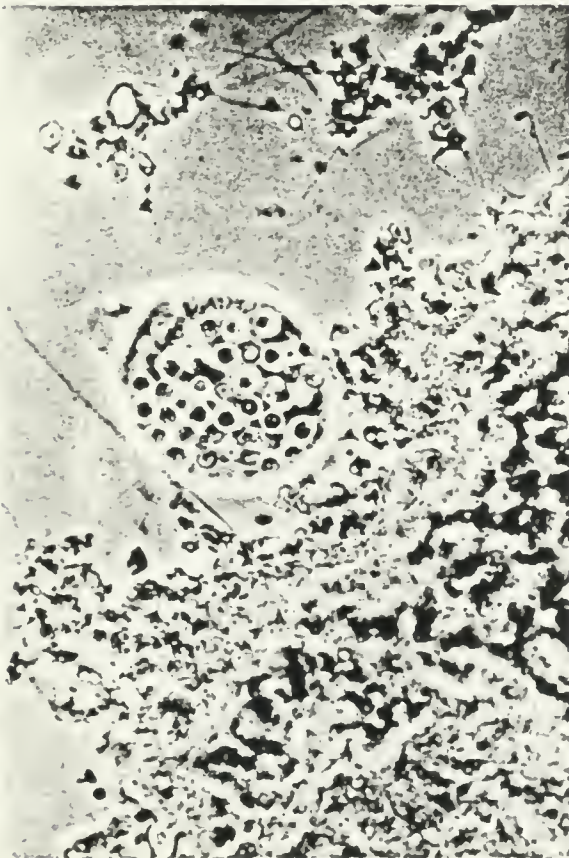


Figure 7-10

Microscopic View of Vorticella  
Organism Dying



Figure 7-11

Escaping Life Processes



SUBJECT:

TOPIC: 8

STATISTICAL ANALYSIS

OBJECTIVES:

The student will be able to:

1. Select the correct definition, from a given list, for each of the following terms.
  - a) Mean
  - b) Standard Deviation
2. Define the term "Line of best fit".
3. Define the term "Method of Least Squares".



## STATISTICAL ANALYSIS

This topic will provide the student with some very basic principles of statistical analysis. It will assist the student in presenting a collection of raw data in a more useful form for inclusion in reports and presentations. For further study of statistical methods and data presentation a number of good reference texts is available; one such reference source is "Modern Elementary Statistics" by John E. Freund, Fifth Edition, Prentice Hall Inc.,

### RAW DATA

Raw data normally consists of a collection of numbers which have not been organized into any meaningful order. Statistical principles allow us to manipulate raw data into a more generalized or more understandable form for inclusion into reports and publications. Use of these principles also simplifies calculations for future statistical work. Examples of routinely collated raw data are Suspended Solids, BOD<sub>5</sub>, Phosphorus, F/M Ratio, pH, Rainfall, flow rates, Chlorine Residuals - etc.

### GROUPED DATA

Grouped data, sometimes referred to as a frequency distribution, is often useful to summarize new data. Grouping presents data in a relatively compact form, and gives an overall picture of the raw data which is often adequate for many purposes. Details, however, are not presented.

#### General Rules for Grouping:

1. Determine the largest and the smallest number in the raw data and thus find the range of the data.

2. Divide the range into a convenient number of classes (intervals or groups), preferably each class should be of equal size. The number of class intervals is usually arbitrarily chosen at between 5 and 20 intervals, depending on the raw data.
3. Determine the number of observations falling into each class or interval. This is best done on a tally sheet.

#### EXAMPLE

The following array of raw data represents a collection of values for some hypothetical parameter (BOD<sub>5</sub>, SS, rainfall, flow rate - etc -).

68	84	75	82	68	90	62	88	76	93
73	79	88	73	60	93	71	59	85	75
61	65	75	87	74	62	95	78	63	72
66	78	82	75	94	77	69	74	68	60
96	78	89	61	75	95	60	79	83	71
79	62	67	97	78	85	76	65	71	75
65	80	73	57	88	78	62	76	53	74
86	67	73	81	72	63	76	75	85	77

TABLE 8-1 RAW DATA

The range of this data is from 53 to 97.

For convenience let us select 10 classes as shown below.

<u>Class Interval</u>	<u>Tally</u>	<u>Number of Observations</u>
50 - 54	53	1
55 - 59	57, 59	2
60 - 64	60, 60, 60, 61, 61, 62, 62, 62, 62, 63, 63	11
65 - 69	65, 65, 65, 66, 67, 67, 68, 68, 68, 69	10
70 - 74	71, 71, 71, 72, 72, 73, 73, 73, 73, 74, 74, 74	21
75 - 79	75, 75, 75, 75, 75, 75, 75, 76, 76, 76, 76, 77, 77, 78, 78, 78, 78, 78, 79, 79, 79	6
80 - 84	80, 81, 82, 82, 83, 84	9
85 - 89	85, 85, 85, 86, 87, 88, 88, 88, 89	4
90 - 94	90, 93, 93, 94	4
95 - 99	95, 95, 96, 97	

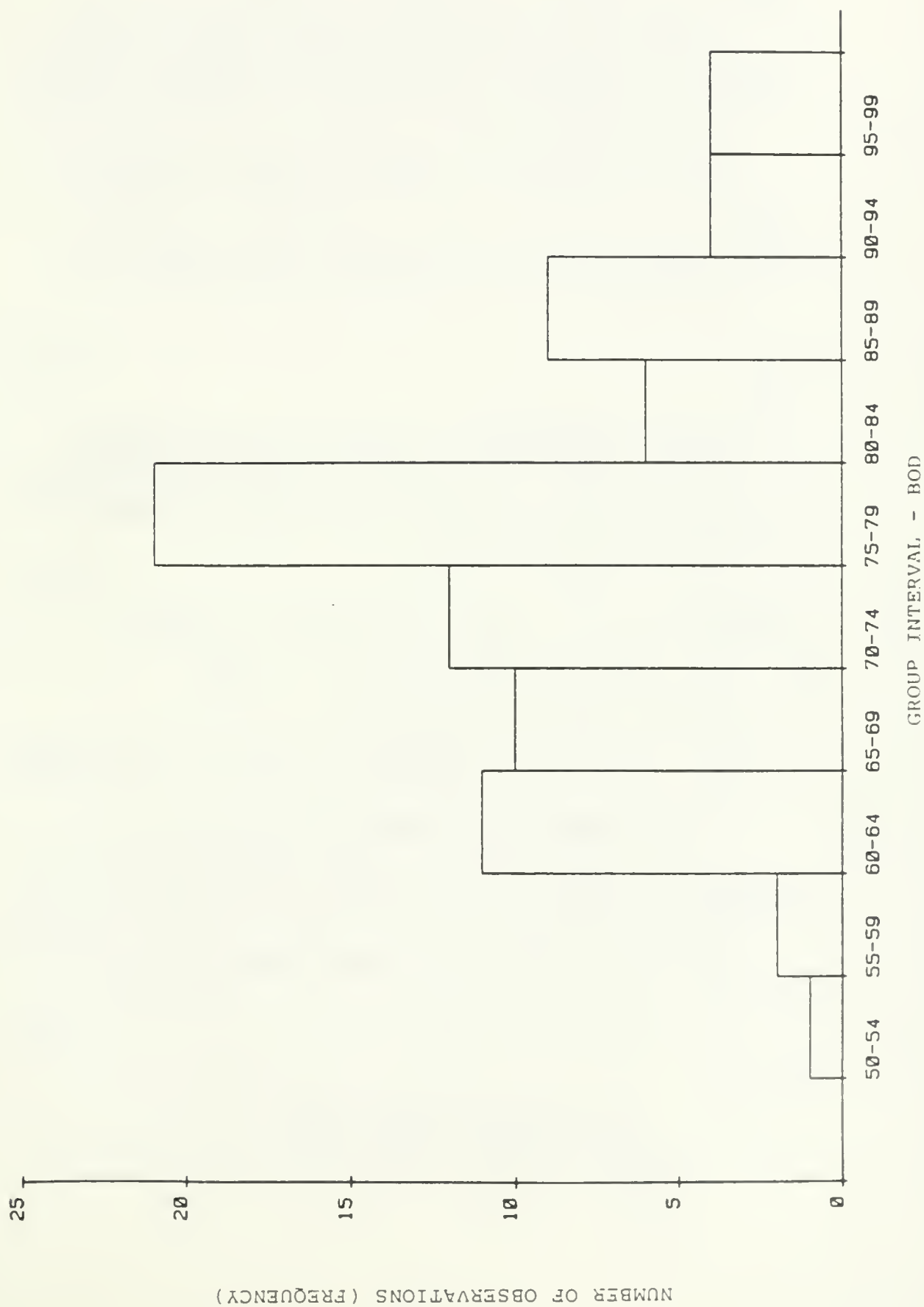


FIGURE 8-1 EXAMPLE OF HISTOGRAM

The grouped data can now be presented in graphical form by means of a HISTOGRAM. A histogram consists of a set of rectangles or stripes. The width of the base of each rectangle represents the class interval or size. The height of the rectangle equals the number of observations (the "frequency") of data falling into that class.

Again, a histogram is used to simplify the presentation of a large collection of raw data. A histogram to illustrate the raw data presented in Table 8-1 and grouped in Table 8-2 is presented as Figure 8-1.

### MEAN

The mean represents the values of a set of numbers with a single value. It may also be known as the average or arithmetic mean. In statistics the mean is normally identified by the symbol  $\bar{X}$ .

#### Example 1

$$\bar{X} = \frac{X_1 + X_2 + X_3 + \dots + X_N}{N} = \frac{\sum X}{N}$$

where  $\sum X$  = sum of all numbers of a given set for which the mean is to be calculated.

N = number of data items in the set

#### Example 2

Find the arithmetic mean of the following set of numbers:

130, 132, 127, 129, 132

#### Solution

$$\begin{aligned}\bar{X} &= \frac{130 + 132 + 127 + 129 + 132}{5} \\ &= \frac{650}{5} \\ &= 130\end{aligned}$$

## WEIGHTED MEAN

The mean is best utilized where all data items have the same importance or weight. If one or more of the items has a measureable importance or weight that differs significantly from the others, a factor can be attached to each item to account for this. The expression for the Weighted Mean now becomes:

$$\bar{X}_w = \frac{X_1W_1 + X_2W_2 + X_3W_3 + \dots + X_NW_N}{W_1 + W_2 + W_3 + \dots + W_N} = \frac{\sum XW}{\sum W}$$

### Example

A company has 80 employees. 60 employees earn \$3.00/hr, 20 earn \$2.00/hr. Find the average hourly rate (ie. the weighted mean)

### Solution

$$\begin{aligned}\bar{X}_w &= \frac{(\$3)(60) + (\$2)(20)}{60 + 20} = \frac{\$220}{80} \\ &= \$2.75\end{aligned}$$

## MEDIAN

The median of a set of numbers arranged in order of magnitude is the middle value, or the arithmetic mean of the two middle values where an even number of items is presented. On a histogram the median would divide the display into two equal areas.

### Example

10, 13, 16, 18, 20 - has a median of 16

3, 4, 4, 5, 6, 8, 8, 8, 10 - has a median of 6

5, 5, 7, 9, 11, 12, 15, 18 - has a median of  $\frac{9 + 11}{2} = 10$

## MODE

The mode is that value in a set of numbers which occurs with the greatest frequency, ie - the most common value. On a histogram the value of the mode represents the highest point (Maximum value).

### Example

2, 2, 5, 7, 9, 9, 9, 10, 10, 11, 12, 18 - has a mode of 9  
3, 5, 8, 10, 12, 15, 16 - has no mode  
2, 3, 4, 4, 4, 5, 5, 7, 7, 7, 9 - has two modes, 4 and 7

## RANGE

The range of a set of items is the difference between the smallest and largest value. The range gives a quick but not necessarily accurate picture of the variability of the data. It gives only the distance between the extreme values but says nothing about the relative dispersion of the remaining items in the set.

### Example

2, 3, 3, 5, 5, 5, 8, 10, 12 - range is  $12 - 2 = 10$   
2, 4, 4, 12, 12, 12, 12, 12 - range is  $12 - 2 = 10$

## STANDARD DEVIATION

Standard deviation is a measure of the average variability (deviation) of a set of data from the mean. Standard deviation is denoted by the symbol "S" and is defined as follows:

$$S = \sqrt{\frac{\sum (X - \bar{X})^2}{N-1}}$$

A more common form for calculating the standard deviation is given by the following formula:

$$S = \sqrt{\frac{N \cdot \sum X^2 - (\sum X)^2}{N(N-1)}}$$

Both formulae give the same result, but the second formula is more convenient to use, as will be illustrated by the following example:

Calculate the standard deviation using both formulae, for the following set of data

12, 7, 9, 5, 4, 8, 17, 2, 11, 14, 13, 9

For convenience we set up the following table:

<u>X</u>	<u>X - <math>\bar{X}</math></u>	<u>(X - <math>\bar{X}</math>)<sup>2</sup></u>	<u>X<sup>2</sup></u>
12	2.75	7.5625	144
7	-2.25	5.0625	49
9	-0.25	0.0625	81
5	-4.25	18.0625	25
4	-5.25	27.5625	16
8	-1.25	1.5625	64
17	7.75	60.0625	289
2	-7.75	52.5625	4
11	1.75	3.0625	121
14	4.75	22.5625	196
13	3.75	14.0625	169
9	-0.25	0.0625	81
$\Sigma$ 111	0	212.2500	1239

Table 8-3 STANDARD DEVIATION

from Table 8-3

$$\bar{X} = \frac{111}{12} = 9.25$$

$$N = 12$$

using formula 1.  $S = \sqrt{\frac{212.25}{11}} = 4.4$

using formula 2. 
$$S = \sqrt{\frac{12(1239) - (111)^2}{12(11)}}$$

$$= \sqrt{\frac{14868 - 12321}{132}}$$

$$= \sqrt{\frac{2547}{132}}$$

$$= 4.4$$

For most applications, approximately 66% of the values from a set of data differ from the mean by less than one standard deviation. Similarly 95% of the values differ from the mean by less than two standard deviations and 99% of the values differ from the mean by less than three standard deviations.

#### GRAPHIC PRESENTATION OF RAW DATA

Graphic presentation is used to simplify presentations of raw data and to illustrate certain trends which may not always be obvious by observations of the raw data.

The following is an example of a graph prepared for the information obtained from the treatment of arsenic waste with ferric chloride. Ferric chloride was dosed as  $\text{Fe}^{+3}$  with the corresponding percentage removal of arsenic.

<u>Y</u>	<u>X</u>
<u>Fe<sup>+3</sup> dosage (mg/l)</u>	<u>% Arsenic Removal</u>
.50	45
100	54
150	78
200	85
250	96
300	99

Table 8-4 ARSENIC REMOVAL  
RELATIONSHIP

The most widely used relationship between the two variables (X - Y) is in the form of a straight line or an equation expressing a linear relationship of the two variables.

The general form of this equation for a straight line is given by:

$$Y = a + bX$$

a and b are numerical constants  
a = Y intercept of the line  
b = slope of the line

When this linear equation is plotted on ordinary graph paper, all points of the X - Y values, which satisfy the equation, will fall on a straight line.

To fit a straight line to data consisting of paired observations of two variables X and Y (% Arsenic removal and Fe<sup>+3</sup> dosage) we can start by plotting the values on ordinary graph paper. In most cases the observed data points do not necessarily fall exactly on a straight line, but they will be reasonably close so that an approximate straight line can be drawn to illustrate a close relationship

between X and Y (Eyeball Line Method). However, by the Method of Least Squares an equation of a straight line can be calculated which provides the best possible fit to the observed data.

The Method of Least Squares requires that the sum of the squares of the vertical deviations (distances) from the points to the line be as small as possible.

The Least Square line is derived as follows:

$$Y = a + bX$$

from the observed data we calculate the "a" and "b" as follows:

$$b = \frac{N(\sum XY) - (\sum X)(\sum Y)}{N(\sum X^2) - (\sum X)^2}$$

$$a = \frac{\sum Y - b(\sum X)}{N}$$

generally "b" is calculated first and then is used in the calculation of "a".

- N = number of observations
- $\sum X$  = sum of the given X's
- $\sum Y$  = sum of the given Y's
- $\sum X^2$  = sum of the squares of the X's
- $\sum XY$  = sum of the products of the corresponding X's and Y's

#### Example

For the calculation of the Least Square Line equation for the arsenic removal data, using data from Table 8-4.

	<u>X</u>	<u>Y</u>	<u>X<sup>2</sup></u>	<u>XY</u>
	45	50	2025	2,250
	54	100	2916	5,400
	78	150	6084	11,700
	85	200	7225	17,000
	96	250	9216	24,000
	99	300	9801	29,700
	<hr/>	<hr/>	<hr/>	<hr/>
SUM (Σ)	457	1050	37267	90,050

We now have

$$\begin{aligned}
 N &= 6 \\
 \Sigma X &= 457 \\
 \Sigma Y &= 1050 \\
 \Sigma X^2 &= 37,267 \\
 \Sigma XY &= 90,050
 \end{aligned}$$

substituting these values into the equation we get:

$$\begin{aligned}
 b &= \frac{6(90,050) - (457)(1050)}{6(37267) - (457)^2} \\
 &= \frac{540,300 - 479,350}{223,602 - 208,849} \\
 &= \frac{60450}{14753} \\
 &= 4.1
 \end{aligned}$$

then

$$\begin{aligned}
 a &= \frac{(1050) - 4.1(457)}{6} \\
 &= \frac{1050 - 1874}{6} \\
 &= \frac{-824}{6} = -137
 \end{aligned}$$

The equation of the Least Square Line is therefore.

$$Y = a + bX$$

$$Y = -137 + 4.1 X$$

a = Y intercept of the line

b = slope of the line

To plot the line on ordinary graph paper, we only have to choose two arbitrary values for X and calculate the corresponding values of Y, plot the two points and through them draw the required line.

#### Example

Arbitrarily choose two available values for X, say 40 and 100. Then,

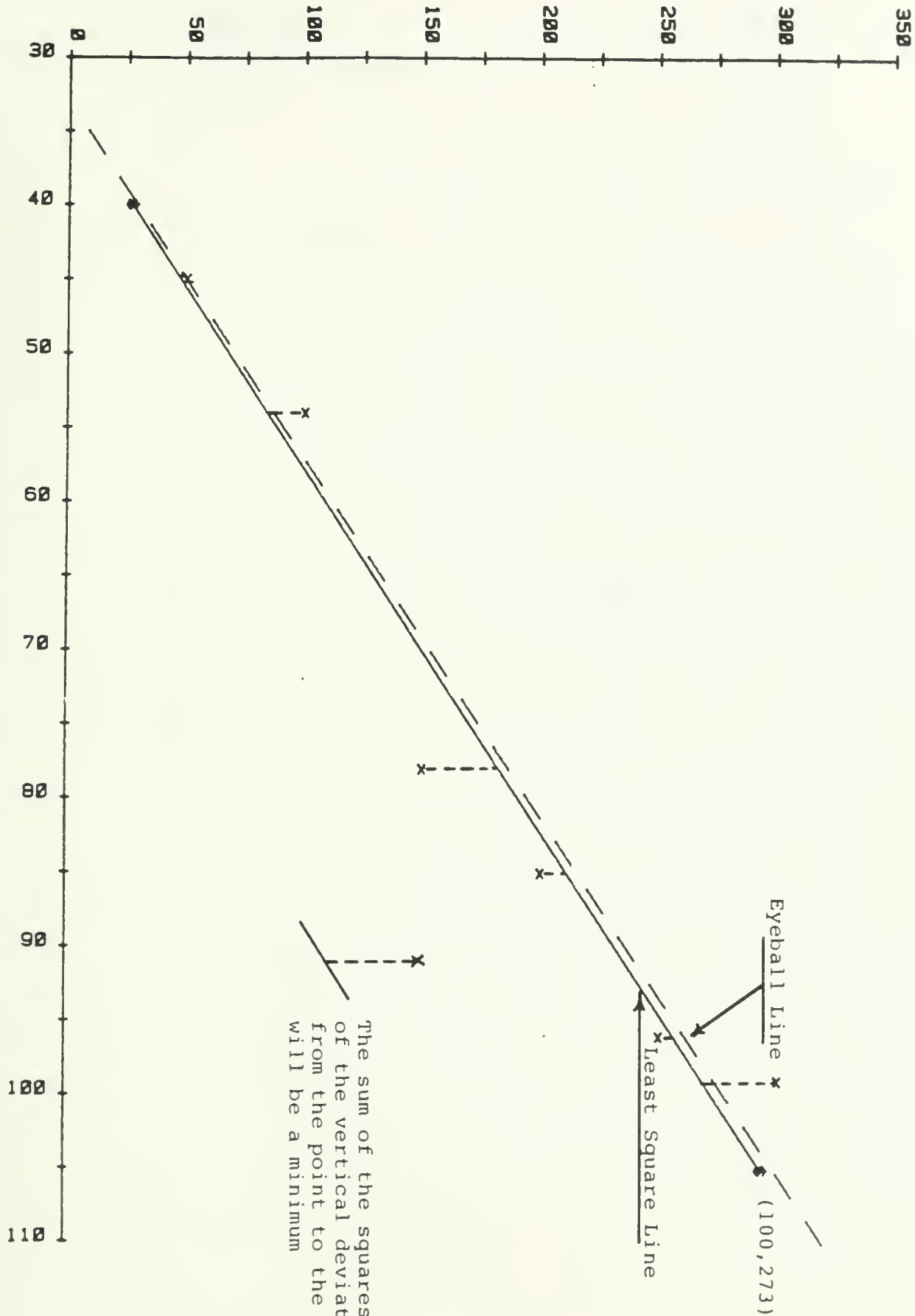
$$Y = a + bX$$

$$\begin{aligned} \text{when } X &= 40, & Y &= -137 + 4.1 (40) \\ & & &= -137 + 164 \\ & & &= 27 \end{aligned}$$

$$\begin{aligned} \text{when } X &= 100, & Y &= -137 + 4.1 (100) \\ & & &= -137 + 410 \\ & & &= 273 \end{aligned}$$

Then the two points are (40,27) and (100, 273). Figure 8-2 shows a plot of the raw data including straight lines drawn by the Eyeball method and the Method of Least Squares.

Fe<sup>+3</sup> DOSAGE mg/L  
 81-8



The sum of the squares of the vertical deviations from the point to the Line will be a minimum

Eyeball Line

Least Square Line

(100, 27.3)

FIGURE 8-2 EXAMPLE OF LEAST SQUARE METHOD OF FITTING STRAIGHT LINE TO DATA

PERCENT ARSENIC REDUCTION



SUBJECT:

TOPIC: 9

LABORATORY SAFETY

OBJECTIVES:

The student will be able to:

1. List the personal requirements for lab safety
2. List six hazards encountered in the laboratory
3. List four rules for good personal hygiene in the lab
4. Describe a safe method for diluting a strong acid
5. List four basic rules for safe handling of glassware



## LABORATORY SAFETY

### INTRODUCTION

Safe operation in a laboratory environment has several basic requirements, including:

1. Attitude
2. Knowledge
3. Experience
4. Equipment
5. Working Habits

#### 1. ATTITUDE

Regardless of the physical aids available, a good attitude toward safety is required. No laboratory operation is completely safe unless the individual has an attitude to safety which promotes the proper application of the other basic requirements. Remember that your co-workers rely on you to act safely to protect them as well as yourself.

#### 2. KNOWLEDGE

A sound knowledge of safe laboratory practices is essential for safe laboratory operation. The individual must have a good working knowledge of the hazards associated with his work and the precautions required to ensure a safe operation. Your supervisor is required by law to acquaint you with any hazards in your workplace (Occupational Health and Safety Act). It is your responsibility to act safely and wear the protective devices supplied. If you are not sure, ask your supervisor.

### 3. EXPERIENCE

Experience in a laboratory operation puts your formal knowledge of safety procedures into practice. There can be no substitute for experience.

### 4. EQUIPMENT

Proper and adequate laboratory equipment is essential for the conduct of safe laboratory practices. This includes your personal safety equipment as well as the laboratory equipment which you use to perform your job. Your employer is required to provide and maintain safety equipment and to ensure that you know the location of the equipment and know how to use it. It is your responsibility to use the equipment provided.

### 5. WORKING HABITS

Good work habits lead to good safety habits. A careful worker is seldom the cause of injury to himself or to his co-workers. Good work habits include the use of safety equipment when required.

### SAFETY AWARENESS

The working environment of the laboratory is by nature a hazardous one. It is only by constant safety awareness on the part of all concerned that the laboratory can be made a safe place in which to work.

The safety legislation of most labour jurisdictions provides that it is the duty and responsibility of the lab supervisor to make each employee aware of the hazards he/she faces and also to provide training in the safety practices required to avoid these hazards. Likewise, it is the duty of every employee to follow safety practices as instructed and

to work in a safe manner. In some instances this is followed to the letter with new employees whose safety awareness is fresh to begin with, but the safety awareness of veteran employees is allowed to become dulled with time.

Under these circumstances, friction can develop between employees on the subject of safety and safe working habits. This situation can be avoided by holding safety tailgate-session at which the safety hazards associated with a designated topic are outlined and the measures used to combat these hazards are discussed. Records should be kept of attendance at "tailgate" safety sessions, the topics discussed, and any suggestions made for improvement in safety practices.

#### HAZARDS IN THE LAB

The hazards associated with the water or wastewater treatment laboratory are essentially the same as those found in other laboratories. In addition to the hazards commonly found in most industrial workplaces, the laboratory frequently contains the following hazards:

##### 1. CUTTING HAZARDS:

Examples of this hazard are situations resulting from:

- a) broken glassware
- b) sharp objects (metal edges, paper)
- c) knives, scissors, needles

A conscious effort must be made to dispose of all broken glassware items and sharp objects in an accepted, safe manner, if this hazard is to be minimized in the laboratory.

## 2. CHEMICAL HAZARDS

The water or wastewater treatment plant laboratory frequently contains chemicals which have hazards associated with their handling. The hazard may be in the form of a gas, mist, vapour, dust or a liquid which is corrosive, flammable, explosive or toxic. Only by knowing the identity of the substance can we effectively protect ourselves from its hazards. The chemical container label is a most valuable source of information concerning the hazards of the substance contained. Recent legislation in most jurisdictions ensures that the hazards associated with a substance are clearly listed. In addition, many chemical supply companies now clearly set out the precautions for safe handling of chemicals on the chemical label. For these reasons, unlabeled chemicals or chemicals in very old containers should be disposed of in a safe, appropriate manner.

## 3. MICROBIOLOGICAL HAZARDS

Both water and wastewater treatment plant laboratories have the potential for microbiological contamination of the laboratory environment. Plates containing bacteria colonies isolated from water samples are one possible source of personal infection. In addition, virtually all samples from wastewater treatment facilities contain living organisms and have the potential to transmit disease. For these reasons, scrupulous personal hygiene must be practiced at all times in the laboratory. Food or drink must never be consumed in any laboratory. In addition, smoking must be prohibited to minimize the potential for personal infection.

#### 4. BURN HAZARDS

Since many laboratory procedures include the use of a flame, hot plate or heating mantle, the potential for heat burns is great in the laboratory. By clearly marking hot surfaces, this hazard can be somewhat reduced. In addition, fire extinguishers and a fire blanket should be kept readily available.

The frequent use of common acid and bases means that the potential for corrosive substance "burns" is always present. The use of easily recognized labels for all corrosive substances, training in the characteristics of corrosive substances and the use of personal protective equipment must be common practice if the incidence of accident from this cause is to be reduced.

#### 5. SAMPLING HAZARDS

Since most water or wastewater treatment plants consist of open tanks with water depths greater than 2 meters, and channels full of fast flowing water, the potential for drowning is always present. In addition, a number of locations in the treatment plant must be considered to be "confined spaces" as defined under the Occupational Health & Safety Act (Ontario), with all the associated hazards. Combining these hazards with the frequent hazard of shaft-driven, electrically operated machinery, results in a work environment with tremendous potential for accident. Sample gathering in the plant should only be undertaken using techniques calculated to minimize the hazards to personnel. No sample is so valuable as to be worth the life of the sampler.

## 6. ELECTRICAL SHOCK HAZARD

Since many of the instruments used in the water or wastewater treatment plant laboratory are electrically operated, the wet environment of the lab creates a most hazardous condition with potential for electrical shock. Many wet-chemistry techniques are best performed in or near the sink, but frequently the procedure requires measurement at some stage, using an electrically-operated instrument of some sort. To safely accommodate both of these requirements it is best to have the laboratory arranged into two distinctly separate work areas: a wet area and a dry area. Measurement using electrically-operated instruments should only take place in the dry area, away from sinks and frequent splash or spill areas. In addition, all electrical devices used in the lab should be powered by a three-prong "u-ground" power cord. The use of a ground fault interrupter is an excellent idea when electrical equipment is operated in a wet environment.

## 7. PERSONAL HYGIENE

Because of the dual hazard of personal infection by pathogenic organisms and poisoning by toxic laboratory substances, all laboratory workers must practice scrupulous personal hygiene if illness is to be avoided.

Microbiological or chemical agents can enter the body through several routes, the most common being ingestion. For this reason a set of common hygiene rules apply to all laboratory practices in water and wastewater treatment plants:

1. No food or drink should be consumed in the laboratory.
2. No smoking in the laboratory.

3. Laboratory items should not be used for food handling.
4. Sample refrigerators should be located in the laboratory and should be clearly marked as such. Food storage refrigerators should not be located in the laboratory area.
5. Lab coats, contaminated gloves and boots, and other personal protective clothing should not be allowed into the lunchroom.
6. Hands and face should be washed before consuming food or smoking.
7. At the end of the shift, lab workers should shower and change into street clothes before leaving the plant.
8. Contaminated or soiled lab clothing should be kept in a separate locker from street clothing.
9. Where substances are known to be hazardous through skin absorption, impervious protective clothing items should be worn.
10. Lab workers should make it a habit to wash their hands frequently with disinfectant soap.
11. Laboratory items should not be removed for use elsewhere. Personal items should not be brought into the lab and then put back into use.

12. Garbage from the lab should be clearly labelled to indicate that a hazard from microbiological or toxic chemical substances exist.

## STORAGE OF CHEMICALS

### 1. QUANTITY

While it is understood that some hazardous substances must be stored in the laboratory, storage must be arranged to minimize the risk to personnel from these primary hazards and secondary hazards created by the inter-action of various stored substances. In addition, the quantities stored should not exceed the quantity expected to be used in the time period needed for replenishment, plus a small quantity to guard against late delivery. In many cases this will be the minimum size available. Discounts achieved by buying bulk orders may seem small when seen in the light of the increased hazard faced by plant staff.

### 2. INTERACTION

Storage should be arranged to minimize the possibility of interaction between reactive chemical substances. For example, oxidizers or acids should not be stored where they can come in contact with substances which can become unstable or explosive in their presence (such as nitrates).

### 3. VOLATILE LIQUIDS

Volatile liquids which are known to produce hazardous fumes which frequently escape from closed bottles, for example, concentrated Hydrochloric Acid should be stored where fumes will be ventilated continuously. An example of this type of storage is a ventilated cupboard below a fume hood.

### 4. FLAMMABLES

Flammable substances should be stored in special cupboards designed for storage of flammable liquids only. If doubt exists concerning the safety of flammables storage, all flammable substances should be stored in ventilated out-buildings with temperature control. In this way, the hazard of injury to personnel and the risk of property damage can both be minimized.

When flammable substances are being handled in the lab, special safety cans should be used for transport and containment. Both grounding and bonding of containers should be practiced when pouring flammable liquids. Again, it is important to limit the quantity of these substances in order to minimize the hazard.

Where flammable substances are used, an accepted spill kit for flammable substances should be kept nearby. The size of this kit should be selected to be sufficient to handle the quantity expected to be stored.

## 5. CORROSIVES

Strong acids and bases should be stored and transported in impact-resistant safety carriers. These substances should be stored below eye level so that the risk of eye damage in a spill is reduced. Impervious protective clothing and safety gear should be worn when handling corrosive substances, eg: face shield, rubber gloves, rubber apron, etc.

Laboratories which use corrosive substances should have a spill kit, for both acids and bases, available nearby. Again the size availability should be sufficient to handle the volume of corrosive substance kept in storage.

## 6. TOXIC SUBSTANCES

When handling toxic substances, rubber gloves should be worn to guard against skin absorption. Quantities of acutely toxic substances kept in storage should be limited, in order to minimize the hazard in the event of a spill or fire. These substances should be kept in secure storage to reduce the possibility of theft or loss through carelessness.

## 7. STORAGE REVIEW

At least once each year, the variety and quantity of chemical substances stored in the laboratory should be reviewed with an aim of removing excess amounts, substances of questionable origin, poorly labelled containers or chemicals acquired for a specific project which is now completed. The saving realized in keeping these items is usually insignificant and the build-up of these substances leads to poor housekeeping habits and crowded storage of needed items.

## PREPARING SOLUTIONS OF STRONG ACIDS & BASES

The dilution of strong acid solutions and the preparation of solutions from solid basic compounds such as NaOH or  $\text{Ca}(\text{OH})_2$  are both exothermic (heat producing) reactions which can be extremely hazardous if performed carelessly. If small amounts of water were to be added to concentrations of either class of substance, great amounts of heat could be produced, resulting in spattering of the corrosive substance by steam and possible injury to the lab worker.

The only accepted safe manner in which water and strong acids can be combined is to start with a relatively large quantity of water and slowly, with stirring, add increments of strong acid to the water, resulting in a dilute solution. The reaction can further be cooled by placing the mixing container in a larger, outer vessel containing a quantity of cooling water. To help in remembering the correct sequence of actions, the following rule is well known in laboratories:

### THE TRIPLE "A" RULE

Always Add Acid - to water

In the same way, solid, strong bases should be slowly added to water in increments with constant stirring. The water bath technique may be used to reduce the time of heat dissipation.

In both cases, the principle is to slowly increase the concentration of the hazardous substance in water, thereby allowing heat to dissipate and reducing the risk of spattering.

Protective clothing should be worn, as well as a face shield, at all times when corrosive substances are being handled.

## 1.DELUGE SHOWERS

Where corrosive substances are commonly used, a deluge shower should be available. A deluge shower is a shower head attached to a water supply pipe of larger than normal diameter for domestic plumbing. The large diameter pipe is capable of supplying large quantities of room-temperature water in a very short time for flushing off corrosive chemical contamination. It should be installed near the entrance to a laboratory so that it can be found very quickly, even by an accident victim who cannot see. In addition to the deluge shower, some laboratories also have an eyewash fountain which spouts two streams of water, spaced eye distance apart. The controlling valve regulates the force of flow and remains open without being held.

When lab workers think they have been splattered by a corrosive chemical, they should immediately make use of the deluge shower with their clothes on and then proceed to remove all of their clothing to flush the skin underneath. Contaminated clothing should not be pulled up over the head, but should instead be torn and removed. A pair of scissors kept nearby will help with tough, knitted fabrics which resist quick removal. Modesty has no place in a spill of corrosives on lab workers. Blankets or borrowed clothing can, afterward, be used to clothe the injured person for transportation.

Where the corrosive has affected the eyes, additional flushing of the eyes for an extended period, using the eyewash fountain, may be required. Continue flushing until the accident victim is in the care of qualified medical attendants.

## 2. FUME HOODS

Where laboratory procedures result in the production of toxic or noxious fumes or vapours, the procedure should be performed in a mechanically ventilated fume hood.

Approved fume hoods should be used and their air flow rate and volume should be measured for verification. Fume hood exhausts should not be connected together except under advice from a competent safety engineer.

When procedures used result in the possibility of bursting apparatus, or flying objects, the safety glass front of the fume hood should not be relied on to give protection. Instead an acrylic, or better still, a Lexan bench-top safety shield should be used to shield lab workers from the procedure.

## 3. GLASSWARE HANDLING

The most frequent injury resulting from lab accidents noted in a good many laboratory safety surveys is a cut from broken glass. Although a great deal of glassware is used in most water and wastewater labs, strict adherence to a few basic glassware handling rules should eliminate this type of injury altogether. The following are some basic rules for handling laboratory glassware:

1. Use only "hardened" glassware items such as Pyrex or Kimex or their equivalent.
2. All broken glassware items should be placed in a carton or other protective covering clearly marked "DANGER: BROKEN GLASS", and disposed of separately from regular garbage.

3. Contaminated broken glass should first be disinfected before disposal.
4. All chipped or cracked glassware should be regarded as broken, and disposed of accordingly.
5. All glass apparatus which is under pressure or vacuum atmosphere should be shielded from lab workers by a safety shield.
6. When rubber and glass items are to be assembled and a force fit is required, the joint should first be lubricated with water and hands should be protected with tough leather gloves further padded by wrapping the joint in rags. The worker's face should also be protected by a face shield during this procedure.
7. The previous precautions should also be taken when cutting glass tubing.

## GLOSSARY OF TERMS

The following definitions are intended only  
as aids in the study of this manual

### ABSORPTION

The taking up of one substance into the body of another.

### ABS

Abbreviation for Sodium alkyl benzene sulfonate.

### ACCURACY

The degree to which a test result agrees with the true value.

### ACID

A compound which yields hydrogen ions in solution.

### ACTIVATED SLUDGE

Sludge floc produced in raw or settled wastewater by the growth of zooglyphic bacteria and other organisms in the presence of dissolved oxygen and accumulated in sufficient concentration by returning of settled floc previously formed.

### ADSORPTION

- 1) The adherence of a gas, liquid or dissolved material on the surface of a solid.
- 2) A change in concentration of gas or solute at the interface of a two-phase system.

### AERATION

- 1) The bringing about of intimate contact between air and a liquid by one or more of the following methods:

- a. spraying the liquid in the air,
- b. bubbling air through the liquid,
- c. agitating the liquid to promote surface absorption of air.

- 2) The supplying of air to confined spaces under nappes, downstream from gates in conduits, etc. to relieve low pressures and to replenish air entrained and removed from such confined spaces by flowing water.
- 3) Relief of the effects of cavitation by admitting air to the section affected.

### AEROBIC

Requiring, or not destroyed by, the presence of free elemental oxygen.

### ALGAE

Tiny plants, usually living in water and often green in colour.

### ALGICIDE

Anything applied to kill or control algae.

### ALIQOT

A sample volume of liquid which is a fractional part of a larger volume.

### ALKALINE

A condition which will raise the pH in water or wastewater higher than 7. Opposite to acidic.

### ALKALINITY

Measure of the content of bicarbonates, carbonates and hydroxide components of a natural or treated water supply.

### ANAEROBIC

Not requiring the presence of free elemental oxygen. Term used particularly with reference to bacteria.

### ANALYSIS

The determination of the composition of a substance.

### ANION

A negatively-charged ion.

### ANODE

The positive plate in an electrolytic system; the plate where oxidation takes place and where electrons are lost.

### ARTESIAN AQUIFER

An aquifer where the water is under pressure and will rise to a higher elevation if afforded an opportunity to do so.

### ATOM

The smallest particle of an element which still retains all the properties of that element.

### ATOMIC NUMBER

The number of protons in the nucleus of an atom.

### ATOMIC WEIGHT

The relative weight of an atom as compared to the weight of some reference atom (commonly carbon 12 = 12.0000).

### AQUIFER

Porous, water-bearing formation of rock, sand or gravel.

### BASE

The hydroxide of a metal. An alkali. A compound which yields hydroxyl radicals in solution.

### BACKFLOW

The backing up of water through a conduit or channel in the direction that is opposite to normal flow.

### BACKWASH

The method used to clean filter media by reversing the water flow.

### BACTERIA

Single-celled microscopic organisms living in soil, water, organic matter or in the bodies of plants or animals.

### BAFFLE

A device to turn aside, check or regulate flow.

### BAR SCREEN

A rack made of parallel bars for removing coarse materials in the wastewater passing through it.

### BEER'S LAW

Absorbance of a solution is proportional to the length of light path and to the concentrate.

### BOD

Biochemical Oxygen Demand - A measure of the oxygen used in decomposing organic matter.

### BOOSTER STATION

A pumping station in a water distribution system, used to increase the pressure in the mains on the discharge side of the pumps.

### BULKING

Bulking occurs in activated sludge plants when the activated sludge becomes less dense and will not settle properly. Often results from the presence of filamentous organisms.

#### CAPILLARY

A tube having a very small inside diameter.

#### CATALYST

A substance which changes the rate of a reaction without itself being consumed in the reaction.

#### CATHODE

The negative plate in an electrolytic system; the plate at which electrons are taken up, and where reduction takes place.

#### CATION

A positively-charged ion.

#### CENTRIFUGE

A machine that separates solids from wastewater in a spinning motion.

#### CHEMICAL CHANGE

A drastic change in properties to the extent that a new substance is formed.

#### CHEMICAL OXYGEN DEMAND

A measure of the oxygen-consuming capacity in inorganic or organic matter present in water or wastewater. It is expressed as the amount of oxygen consumed from a chemical oxidant in a specific test. It does not differentiate between stable and unstable organic matter and thus does not necessarily correlate with biochemical oxygen demand. Also known as OC and DOC, oxygen consumed and dichromate oxygen consumed, respectively.

#### CHLORINE DEMAND

The difference between the amount of chlorine added to a water or wastewater and the amount of chlorine residual left after a certain length of time.

### CHLORINE RESIDUAL

The amount of chlorine still left available after a certain length of contact time.

### CLARIFIER

A unit of which the primary purpose is to cause clarification. (settling of solids to produce a clear supernatant). Usually applied to sedimentation tanks or basins.

### CLEAR WELL

Reservoir for storing treated, filtered water.

### COAGULANTS

In water and wastewater, chemicals used to cause coagulation of finely divided suspended solids into larger particle groups for easy removal.

### COAGULATION

In water and wastewater treatment, the destabilization and initial aggregation of colloidal and finely divided suspended matter by the addition of a floc-forming chemical or by biological processes.

### COLIFORM

A group of bacteria which normally live in the intestines of man and animals and are also found elsewhere in nature. They are pollution indicators in water supplies.

### COLLOIDAL

Too finely divided to settle; requiring coagulation, biochemical action, or membrane filtration for removal.

### COMBINED CHLORINE RESIDUAL

The concentration of chlorine combined with ammonia as chloramine or as other chloro-derivatives, yet is still available to oxidize organic matter to carry on disinfection of water.

#### COMMINUTOR

A device for reducing solids to minute particles. Trade name for a shredding device used in pre-treatment of wastewater.

#### CONTACT BASIN

A basin used to put water or wastewater in contact with chemicals or other materials; for example a chlorine contact chamber.

#### CONTAMINATION

The presence of micro-organisms, chemicals or wastes that make water unfit for use.

#### DECOMPOSITION

Generally aerobic processes that convert unstable materials into more stable forms by chemical or biological action. Waste treatment encourages decay in a controlled situation in order that the material may be disposed of in a stable form. When organic matter decays under anaerobic conditions (putrefaction), undesirable odours are produced. In aerobic processes, the odours are much less objectionable than those produced by anaerobic decomposition.

#### DENSITY

The mass per unit volume of a substance.

#### DESICCANT

Drying agent; absorbs moisture from its surroundings.

#### DETENTION TIME

The length of time that wastewater is held in a unit for treatment.

#### DIATOMACEOUS EARTH

A fine soil made up mostly of the skeletal remains of diatoms.

#### DIATOMS

Single-celled microscopic algae that grow in or on water and have skeletons of silica.

### DIFFUSER

A device for distributing tiny air bubbles throughout a liquid, such as wastewater.

### DIGESTION

The biological decomposition of organic matter to a more stable form.

### DISINFECTION

Destruction of disease-causing micro-organisms by physical or chemical means (chlorination or boiling of water).

### DISSOLVED OXYGEN

Atmospheric oxygen dissolved in water or wastewater, usually abbreviated DO.

### DISTILLATE

The condensed liquid obtained by a distillation process.

### DISTRIBUTION SYSTEM

A system of piping, canals, and associated equipment used to distribute a water supply to consumers.

### EFFLUENT

In wastewater treatment, wastewater or other liquid flowing out of a reservoir, basin, treatment plant, or industrial treatment plant.

### ELECROLYTE

A substance which will conduct a current when melted or in solution.

### ELECTRON

A negatively-charged atomic particle.

### ELUTRIATE

To purify, separate or remove by washing.

### ENDOGENOUS

A diminished level of respiration in which materials previously stored by the cell are oxidized.

### ENZYME

A protein that promotes a chemical reaction, enabling it to continue at body temperature.

### EQUILIBRIUM

Condition in which two processes proceed simultaneously in opposite directions at the same rate.

### EXCESS

An amount of a reactant greater than that required to cause completion of a reaction.

### FILAMENTOUS BACTERIA

These bacteria develop where carbohydrates are present and where there is low dissolved oxygen content. The result is bulking and poor setting. These organisms grow in a thread of filamentous form.

### FILTER CAKE

Dewatered sludge or sediment.

### FILTER MEDIUM

The material through which water or wastewater is filtered.

### FILTRATE

The liquid obtained from filtration.

### FILTRATION

The process of passing a liquid through a filter to remove suspended solids.

## FLOC

Small jelly-like masses formed in a liquid by adding a coagulant or as a result of microbiological secretion.

## FLOCCULATION

The collection of coagulated suspended solids into a mass by gentle stirring.

## FLOCCULATION AIDS

Materials added to liquid to form floc.

## FLOCCULATOR

Mechanical equipment used to encourage the formation of floc in liquid.

## FLOTATION

The raising of suspended matter to the surface of wastewater in a tank for removal by skimming.

## FORMULA

The composition of a substance indicated by symbols of each element present and subscript numbers showing the number of each type of atom present.

## FORMULA WEIGHT

The sum of the atomic weights of all atoms in a formula; molecular weight.

## FUNGI

Small non-chlorophyll-bearing plants which lack roots, stems or leaves, which occur (among other places) in water, wastewater or wastewater effluents and grow best in the absence of light. Their decomposition after death may cause disagreeable taste and odours in water; in some wastewater treatment processes they are helpful and in others they are detrimental.

### GRAM ATOMIC WEIGHT

The atomic weight of an element expressed in grams to indicate the proportions by which an element will react with other elements.

### GRAM MODULAR WEIGHT

The molecular weight of a compound expressed in grams to indicate the proportions by which a compound will react with other compounds.

### HYDROLOGIC CYCLE

The movement of water from the atmosphere to the earth and back to the atmosphere through precipitation, infiltration, storage, transpiration, evaporation etc.

### HYDROLYSIS

A chemical process of decomposition using the addition of water. Also, the process solid matter goes through to become liquid.

### INDICATOR BACTERIA

Coliform bacteria that point to the presence of intestinal pathogens in contaminated water.

### INFLUENT

Water or wastewater flowing into a treatment plant or any of its units.

### INORGANIC

Made of matter that is not plant or animal in origin.

### ION

An electrically-charged atom.

### ION EXCHANGE

A chemical process in which ions from two different molecules are exchanged.

### IONIZING

Creating ions by adding electrons to, or removing them from atoms.

### IRON BACTERIA

Bacteria that use iron as food and discharge its compounds in their life processes.

### ISOTOPES

Atoms which have the same number of protons but different numbers of neutrons in their nuclei.

### LEACHING

Percolating liquid through soil or other solids to remove the soluble ingredients.

### MENISCUS

The curve formed by the free surface of a liquid in a column.

### METABOLISM

The process in which food is used and wastes are formed by living matter.

### METAL

Element which readily loses electrons to form positive ions.

### MF

Membrane Filter, test method (used in bacteriological lab test).

### MICROBES

Microscopic organisms, micro-organisms, especially pathogenic bacteria.

### MICRO ORGANISMS

Minute organisms, either plant or animal, invisible or barely visible to the naked eye.

### MIXED LIQUOR

A mixture of activated sludge and organic matter undergoing treatment in the aeration tank of a wastewater treatment plant.

### MIXTURE

A combination of substances held together by physical rather than by chemical means.

### MOLALITY

The number of gram molecular weights of solute per 1000 grams of solvent.

### MOLARITY

The number of gram molecular weights of solute per litre of solution.

### MOLECULAR WEIGHT

The relative weight of a molecule as compared to the weight of some reference atom (commonly carbon 12 = 12.0000).

### MOLECULE

The smallest particle of a compound which still retains all the properties of that compound.

### MPN

Most Probable Number, test method (used in bacteriological lab test).

### NEUTRALIZATION

Change in pH toward neutral position (7); reaction of an acid and a base to form a salt and water.

### NEUTRON

An uncharged nuclear particle with a mass approximately equal to that of a proton.

### NITRIFICATION

The conversion of ammonia into nitrates by bacteria.

### NORMALITY

The number of gram equivalent weights of solute per litre of solution.

### NUTRIENT

Food for the growth of organisms.

### ORGANIC

Made of matter that is plant or animal in origin.

### OXIDATION

The act of combining with oxygen; any reaction which involves the loss of electrons from an atom. A positive change in valence.

### OXIDIZING AGENT

A substance that oxidizes another substance.

### OZONATION

The act or process of charging or treating with ozone. Also, the conversion of oxygen into ozone. Used for disinfection purposes.

### PARSHALL FLUME

A device used to measure liquid flow in a channel.

### PATHOGENIC

Disease-producing bacteria.

#### PERMEABLE

Having pores or openings that permit liquids or gases to pass through.

#### pH

The measure of the acidity/alkalinity balance, expressed on a scale of 0 to 14, with 7 being neutral; 7 to 0 increasing acidity, and 7 to 14 increasing alkalinity.

#### PREAERATION

A method of preparing wastewater for treatment by aeration to remove gases, add oxygen, float grease, etc.

#### PRECIPITATE

A solid which forms in and settles out from a solution.

#### PRECISION

A measure of the degree of reproducibility of a test value.

#### PRESSURE HEAD

A measure of the pressure exerted by a fluid.

#### PROTON

A positively-charged nuclear particle.

#### PROTOZOA

Unicellular microscopic animals. They feed on the surface of biological floc and on dispersed bacteria, which results in a clear effluent. Protozoa are used as indicator organisms in troubleshooting of the Activated Sludge process.

#### PSEUDOMONAS

Short rod-shaped bacteria, some of which live on dead or decaying organic matter, or cause disease.

### PUTRESCIBLE

- 1) The relative tendency of organic matter to undergo decomposition in the absence of oxygen.
- 2) The susceptibility of wastewaters, effluents, or sludge to putrefaction.

### RADICAL

A group of elements bonded together, possessing an electrical charge which behaves chemically like a single atom.

### REACTION

A chemical change.

### REDUCING AGENT

A substance that causes the loss of an electron.

### REDUCTION

A gain of electrons; a negative change in valence.

### RETENTION TIME

Detention time; the length of time that water or wastewater is held in a unit for any treatment.

### ROTIFERS

Multicellular microscopic animals which feed on bacteria and protozoa. They are normally found in extended aeration systems.

### SALT

A compound which ionizes but which produces neither hydrogen ions nor hydroxyl radicals in solution.

### SEDIMENTATION

Settling or clarification; the process of allowing solids in water and sewage to sink to the bottom for easy removal.

### SEPTIC

Anaerobic (decomposition without oxygen).

### SOLUTION

Homogeneous, non-settling mixture of two ingredients, solute and solvent.

### SPECIFIC GRAVITY

The ratio of the density of any substance to that of some reference substance. For solids and liquids, water is the standard substance; for gases, air is the standard substance.

### SPORES

Walled, single-to-many-celled reproductive bodies of micro-organisms, able to produce new organisms directly or indirectly.

### STABILIZE

To convert to a form that resists change. Organic material is stabilized by bacteria which convert the material to gases and other relatively inert substances. Stabilized organic material generally will not give off obnoxious odours.

### STAINING

Colouring specimens for microscopic study. Also, colouring or discolouring anything.

### STANDARD SOLUTION

Solution of accurately-known concentration.

### SUPERNATANT

The liquid standing above a sediment. In sludge digestion, the liquid standing between the sludge at the bottom and the scum at the top.

### SURFACE WATER

All water found on the surface of the earth.

### SUSPENDED SOLIDS

- 1) Solids that either float on the surface of, or are in suspension in water, wastewater or other liquids, and which are largely removable by laboratory filtering.
- 2) The quantity of material removed from wastewater in a laboratory test, as prescribed in "Standard Methods for the Examination of Water and Wastewater" and referred to as non-filterable residue.

### TITRATION

The method of finding how much of a substance is in a solution by measuring how much of another solution is needed to complete a chemical reaction.

### TOTAL SOLIDS

The sum of dissolved and undissolved constituents in water or wastewater, usually stated in milligrams per litre. Solids left behind on evaporation of water or wastewater samples.

### TRANSPIRATION

The process by which plants return water to the atmosphere.

### TURBIDITY

A condition in water caused by suspended matter; murkiness.

### VALENCE

The relative combining capacity of an element. A number indicting the charge on an ion or the number of pairs of electrons shared by one element with another.

### VOLATILE SOLIDS

The quantity of solids in water, wastewater or other liquids, lost on ignition of the dry solids at 550 degrees C. The quantity of organic solids in a sample.

### WEIR

A dam or enclosure in water or wastewater used to raise the water level or change the direction of its flow; with notches or a crest, it measures the flow.





